SCIENTIFIC CRITERIA DOCUMENT

FOR THE DEVELOPMENT OF

A PROVINCIAL

WATER QUALITY OBJECTIVE

FOR

BACILLUS THURINGIENSIS VAR. ISRAELIENSIS (*Bti*)

DRAFT

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PREFACE

The Ontario Ministry of Environment develops Provincial Water Quality Objectives or Interim Objectives for those substances deemed to be of environmental concern in Ontario as determined through a screening process which considers persistence, potential to bioaccumulate, acute and chronic toxicity and potential presence in the aquatic environment. Alternatively, Ministry staff who have a direct responsibility for managing the possible effects of these chemicals and/or biorational substances may request an evaluation. Biorational substances have properties of a toxic chemical that permit their use against a pest species without affecting important non-target species. Furthermore, they decompose into harmless compounds following application (Joung and Cote 2000). The biorational substance that is detailed below is the micro-organism *Bacillus thuringiensis* var. *israelensis* (*Bti*).

Provincial Water Quality Objectives and Interim Objectives (PWQO/IPWQO) are numeric or narrative criteria intended to protect all life stages of aquatic organisms from indefinite exposures and/or to protect recreational uses of water. PWQO/IPWQO for recreational uses, including swimming, are currently based only on microbiological and aesthetic considerations. The potential for harmful effects from recreational exposure to chemical substances is assessed on a case by case basis. Ontario Drinking Water Standards and sport fish consumption guidelines for the protection of human health are also considered.

PWQO/IPWQO represent a desirable water quality for the protection of designated uses of

surface waters in Ontario. Objectives/Interim Objectives do not take into account analytical detection or quantification limits, treatability or removal potential, socio-economic factors, natural background concentrations, or potential transport of contaminants among air, water, and soil. These factors are considered in policies and procedures which govern the uses of PWQO/IPWQO. They are contained in the document, *Water Management: Policies, Guidelines, Provincial Water Quality Objectives of the Ministry of the Environment and Energy* (OMOEE 1994), which deals with all aspects of Ontario's water management policy.

The process for deriving these criteria is detailed in *Ontario's Water Quality Objective Development Process* (1992). The toxicity literature is reviewed in all of the following areas: aquatic toxicity, bioaccumulation, mutagenicity, and aesthetic considerations. The final Objective/Interim Objective is based on the lowest effect concentration reported for any of these factors on aquatic organisms as well as on taste and odour considerations of the water. Where there are reliable and adequate data, an Objective is developed using a safety factor. Where there are fewer data, an Interim Objective is developed using an "uncertainty factor".

The size of the uncertainty factor reflects the availability of appropriate data and the potential of the material to bioaccumulate. The use of this uncertainty factor in Ontario's procedures sometimes produces Interim Objectives that are considerably lower than those of other agencies that do not use this approach. Interim Objectives can be promoted to Objectives when sufficient reliable data become available.

PWQO/IPWQO are used to designate surface waters of the Province which should not be further degraded. They are also used in reviewing water discharge assessments and may be included in Certificates of Approval which are issued to regulate effluent discharge. Where better water quality is required to protect other beneficial uses of the environment in a given location, appropriate criteria and factors, including public health considerations, are taken into account.

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Units of measure

m	metre
cm	centimetre
mm	millimetre
d	day
h	hour
kDa	kiloDalton

Concentrations in this document are expressed in a number of different units commonly used in scientific papers. These units are:

1 gram per litre (g/L) = 1000 milligrams per litre (mg/L)

1 mg/L (or parts per million) = 1000 micrograms per litre (ug/L)

1 ug/L (or parts per billion) = 1000 nanograms per litre (ng/L)

Similarly,

1 milligram per kilogram (mg/kg, or parts per million) = 1000 ug/kg (or parts per billion)

nmoL/mL = nanogram molecular weight per millilitre of solution

Table 1. Conversion table for *Bacillus thuringiensis* var. *israelensis* products when added to water in the field or in the laboratory.

When the rate of application of a product is:	1 kg/ha = 1L/ha	over an area with a depth of:	1.2 m	then the effective concentration of the product in water is:	0.083 ppm
	1 kg/ha = 1L/ha		1 m		0.1 ppm
	1 lb/acre		1 m		0.089 ppm
	1 kg/ha = 1L/ha		0.4 m = 1.3 ft		0.25 ppm
	1 kg/ha = 1L/ha		0.305 m = 1ft		0.328 ppm
	1 kg/ha = 1L/ha		0.1 m		1 ppm
	1 lb/acre		1 ft		0.292 ppm

1.0 INTRODUCTION

The Ontario Ministry of the Environment (OMOE) has the mandate to develop and revise Policies, Guidelines, and Provincial Water Quality Objectives to protect the Province's water resources. The Policies and Guidelines are designed to assure that "...surface waters in the Province are of a quality which is satisfactory for aquatic life and recreation." (OMOE 1992b, OMOEE 1994). Numerical and Narrative Objectives/Interim Objectives represent an essential guide for the protection and maintenance of water quality and may form the basis of chemical-specific effluent limits.

In recent years, the toxicity of pesticides to non-target terrestrial and aquatic biota has caused much public concern and has led to the exploration of more target-specific chemicals. Alternative products to traditional chemical pesticides have been developed that pose fewer risks and/or hazards. One commonly used alternative is a group of microbial insecticides that contain micro-organisms or their by-products. Microbial pesticides are especially valuable because their toxicity to non-target organisms and humans is especially low.

Advantages of Microbial Insecticides

1) Microbial insecticides focus on target pests and are essentially non-toxic and non-pathogenic to wildlife, humans, and other non-target organisms (Weinzierl *et al*. 1998).

2) Microbial insecticides are specific to a single group or species of insect and do not directly

affect beneficial insects in treated areas (Weinzierl *et al*. 1998).

3) Microbial insecticides can be used in conjunction with synthetic chemical insecticides without being deactivated or damaged (Weinzierl *et al*. 1998).

Disadvantages of Microbial Insecticides

1) Heat, desiccation (drying out), or exposure to ultraviolet radiation reduces the effectiveness of several types of microbial insecticides. Thus, the proper timing and application procedures of the biocides are especially important (Weinzierl *et al*. 1998).

2) Microbial pesticides are also known to act as biological pathogens and biological control agents.

3) Bacterial insecticides must be eaten to be effective; they are not contact poisons.

The purpose of this report is to review and evaluate the environmental impacts of the bacterial insect pathogen, *Bacillus thuringiensis* var. *israelensis* (*Bti*) in order to develop a PWQO for the protection of aquatic life. *Bacillus thuringiensis* var. *israelensis* is a biopesticide that is identified as a substance of environmental concern due to its widespread addition to standing water and other breeding sites (e.g., storm water retention areas, ditches, ponds) for the purpose of

selectively reducing populations of mosquito larvae (target organisms) which are potential vectors of the West Nile virus.

1.1 Sources and Uses

There are at least 31 recognized subspecies (serotypes, varieties) (DSMZ 1994) and 800 strain isolates (de Barjac 1981) of *Bacillus thuringiensis.* Any documentation associated with *Bt* must include both the serotype and strain being used as well as a potency comparison to an accepted international reference standard (Surgeoner and Farkas 1990). The products and potencies listed in Table 2 were used in the toxicity studies cited in this document. The potency of the different commercial products are expressed in International Toxic Units per milligrams. For example, 15,000 ITU/mg represents the assigned international potency to which all batches of *Bti* products are compared. In addition to potencies, there are several types of formulations of *Bti* including liquids, pellets, and briquettes. Formulation can determine persistence and thus efficacy (Glare and O'Callaghan 1988).

Table 2. *Bacillus thuringiensis* var. *israelensis* (*Bti*) brand names cited in toxicity papers listed in the bibliography (Reference numbers are listed in Table 4). AS represents aqueous suspension, CG = corncob granules, FC = flowable concentrate, TP = technical powder, WDG = water dispersible granules, WG = wettable granules, WP = wettable powder, PP = primary powder.

Commercial Products	ITU/mg	Reference number
IPS 82 (H-14)	15,000	3
BMP 144 2X	1,200	10
Vecto Bac CG	200	16
Vecto Bac G	200	23
Vecto Bac 12AS	1,200	29
Vecto BAC WP	2,000	20
Vecto Bac WDG	2,700	30
Vecto Bac WG	3,000	29
Vecto Bac TP	5,000	16
Bactimos FC	1,200	17
Bactimos WP	3,500	20
Bactimos PP	9,000-10,500	13
Bactimos PP	10,000 or 6,500	30
Teknar FC	1,500	20
Teknar HP-D	1,500 AAU/mg	4
Teknar HP-D FC	3,000	24
Bti from Abbott	600	19
Bti WP (ABG-6108)	1,000 from Abbott	

Bacillus thuringiensis (*Bt*) was first isolated from diseased silkworm larvae in Japan in 1901 (Surgeoner and Farkas 1990; Priest 1992; Swadener 1994). In 1911 this strain of bacteria was isolated from diseased flour moths in Thuringia, Germany and given taxonomic status as *Bt*. It was first used as a commercial insecticide in France in the late 1930's and in the USA in the 1950's. In the 1960's different strains were discovered to be highly pathogenic to different types

(Orders) of insects e.g., Lepidoptera (butterflies and moths), Diptera (mosquitoes, black flies, fungus gnats) and Coleoptera (beetles). Many *Bt* strains that contain mixtures of up to six or eight different Cry (crystalline) proteins have been widely used as pesticides since the early 1960's. Production of the strain *Bacillus thuringiensis* var. *israelensis* (*Bti*) began in the early 1980's (Boisvert and Boisvert 2000).

1.2 Using *Bt* Insecticides

Since each *Bt* strain controls only certain types of insects, it is essential to identify the target pest and to confirm that the *Bt* product label states that the insecticide is effective against that pest. (Glare and O'Callaghan 1998). The bacteria subspecies have the same basic delta-endotoxins but differ in crystal structures. This results in differential susceptibility of the hosts, likely due to different degrees of binding affinity to the toxin receptors in the insect gut.

When a susceptible insect ingests *Bti*, the protein toxin is activated by alkaline (~ pH 10) conditions and enzyme activity in the insect's gut (Glare and O'Callaghan 1998; Joung and Cote 2000; Boisvert and Boisvert 2000; Lacey and Siegel 2000). The toxicity of this activated toxin is dependent on the presence of specific receptor sites on the insect's gut wall. The necessary match between toxin and receptor sites determines the range of insect species killed by each *Bt* subspecies and isolate. If the activated toxin attaches to receptor sites, it paralyzes and destroys the cells of the insect's gut wall, allowing the gut contents to enter the insect's body cavity and

bloodstream. Poisoned insects may die quickly from the activity of the toxin or may die within 2 or 3 days from the effects of septicaemia (blood poisoning). Although it may be a few days before the insect dies, it stops feeding soon after *Bti* ingestion.

The *Bti* subspecies kills larvae of certain Diptera (the insect order of true flies containing one pair of wings). The main targets for *Bti* are the larval stages of mosquitoes, black flies and fungus gnats; it does not control larval stages of evolutionarily more developed flies such as the house fly, stable fly or blow flies. Mosquitoes that are the most susceptible to *Bti* include species within the genera *Aedes* and *Psorophora*. *Anopheles* and *Culex* species are controlled at higher rates of *Bti* application (Glare and O'Callaghan 1998).

Bti products are formulated for spray or granular applications. *Bti* formulated on corn cob granules is effective against mosquito larvae developing in tires and other artificial containers. The corn cob granules can be blown into tire piles to provide good penetration and uniform treatment; residual control is also greater when corn cob granules are used. *Bti* is not very effective in turbid water or waters containing high levels of organic pollutants (Lee *et al*. 1996; Batra *et al*. 2000; Siegal and Novak 1999)

1.3 Properties and Fate

Bacillus thurigiensis (*Bt*) is a gram-positive bacterium, which is widely distributed in the environment (Young et al. 1998). *Bt* forms asexual reproductive cells called endospores, which

enable them to survive in adverse conditions. This bacterium has been isolated from moist soil (Martin & Travers 1989), tissues of infected insects (AlFazairy 1986; Ghassemi *et al*. 1981), insect habitats (Brownbridge and Margalit 1986), stored products, and decaying leaves of some plants (Smith and Couche 1991) and aquatic environments (Thanabalu *et al*. 1992). However, its role in the aquatic environment remains uncertain (Morris-Coole 1995).

When spores of the species are produced, a proteinaceous parasporal crystalline inclusion (delta-endotoxin) is formed. These inclusion crystals consist of proteins (referred to as Cry proteins), which are selectively active against a narrow range of insects (Hofte and Whitely 1989). These Cry proteins are protoxins that are proteolytically (hydrolytic breakdown of proteins into simpler, soluble substances such as peptides and amino acids) activated upon ingestion, bind to specific sites (i.e., receptors) in the midgut cells of susceptible insects and form ion-selective channels in the cell membrane (English and Slatin 1992). The cells swell due to the influx of water, which leads to cell lysis and, ultimately, death of the insect (Knowles and Ellar 1987). The ultrastructural changes in the midgut epithelial cells in *Culex quinquefasciatus* exposed to *Bti* resulted in microvilli appearing to bubble followed by a total collapse in the cytoplasm (Poopathi et al. 1999).

This species is differentiated internally on the basis of flagellar (H) antigens (de Barjac and Bonnefoi 1962; de Barjac 1981). More than 50 servors (Lecadet *et al.* 1994), which are sometimes considered to represent subspecies (Rabinovich et al. 1995), have been recognized within the species. Servors are groups of closely related micro-organisms distinguished by a

characteristic set of antigens. They are also called serotypes.

Bt is a naturally-occurring pathogen that readily decomposes in the environment (Extoxnet 1996). It is moderately persistent in soil. Its half-life in suitable conditions is about 4 months. The spores of this pathogen are released into the soil from decomposing dead insects that have been killed by *Bt*. *Bt* becomes rapidly inactive in soils that have a low pH below 5.1 and is classified as immobile because the spores do not move or leach in groundwater. The rapid breakdown and low toxicity of *Bt* results in no threat to groundwater. In water *Bt* can be effective up to 48 hours after which the spores gradually settle out or adhere to suspended organic matter. The endotoxin crystals remain toxic for longer periods of time (WHO 1999; Joung and Cote 2000; Boisvert and Boisvert 2000).

Bt is short-lived on vegetation because ultraviolet light rapidly destroys this pathogen (WHO 1999). Its half-life under normal UV exposure is 3.8 hours (Dunkle and Shasha 1989).

Bti is not a contact insecticide and has to be ingested by mosquito larvae to produce toxic effects. It is considered to be the safest for the environment because of its specificity for some Nematocera (sub-order of Diptera), particularly simuliid black flies, mosquitoes, and, to some degree, chironomid midges (Ali 1981; Merritt *et al.* 1989; Becker *et al*. 1992). The biocide is compatible with integrated pest management (IPM) programs because this bacterium is readily available and highly toxic to target pests. An advantage of *Bti* is that it does not consist of

molecules synthesized by man, which usually have a very high persistence in the environment. Naturally occurring *Bt* is found throughout the world in many different habitats.

1.4 Resistance

Resistance is defined as a reduction in the sensitivity of an insect population. It refers to a situation when a product (e.g. *Bti*) repeatedly fails to reach the expected level of control (IRAC 2006) even when it is used according to label instructions for that pest species. Some resistance to the insecticide *Btk* in Lepidopteran pests has been reported (Van Rie *et al.* 1990; Marrone and Macintosh 1993, cited in Glare and O'Callaghan 1998). The development of a low level of resistance in mosquito larvae exposed to *Bti* has also been reported (Georgiou and Wirth 1997) but it is relatively less pronounced than chemical pesticides. Becker and Margalit (1993) detected no difference in resistance in populations of *Aedes vexans* to constant *Bti* exposure in the laboratory for 10 years relative to unexposed populations. One study was done in which 6 to 11 times more resistance was recorded after 27 generations of *Culex pallens pallens*. However, 50% of the subsequent generations of mosquitoes that developed resistance returned to no resistance after three generations (Han 1988; Glare and O'Callaghan 1998). New studies on resistance are summarized by Stark (2005).

Resistance was linked to a single crystalline protein used for mosquito control (Becker and Margalit 1993). In another laboratory study, resistance within a few generations of exposure was also linked to a single *Bti* toxic protein (Georgiou and Wirth 1997); however, the latter authors

concluded that resistance would be more difficult to achieve in mosquitoes exposed to mixtures of endotoxins. (Up to 4 crystalline toxins commonly occur in commercial *Bti* products, see Weir 2005.) Thus, the low level of resistance in mosquitoes to *Bti* may be due to exposure to combinations of up to 4 crystalline proteins (Becker and Margalit 1993; Glare and O'Callaghan 1998). Bauer (1995) provides an overview of resistance management strategies for target organisms to different strains of *Bt*.

1.5 Detection Limits and Ambient Concentrations

Bioassays rather than analytical procedures are used to determine potency of *Bti* products because the active toxic ingredient(s) are comprised of several different proteins. Potency standards are derived from bioassays and assigned International Toxicity Units (ITU) because counts of bacterial spores do not provide accurate standards.

The reference standard (designated IPS 82) maintained by the Pasteur Institute was derived from *Bacillus thuringiensis* var. *thuringiensis* and was given an arbitrary potency of 15,000 ITU/mg (Thiery and Hamon 1998). The ITU/mg is a measure of the potency for the technical material, whereas the ITU's per litre (which equals concentration in ppm x potency) is a measure of toxicity of the product used in the spray programs. Potency is based on the ratio between the LC_{50} of the standard and the LC_{50} of the product in question.

The potency and larvacidal activity of the standard reference powder IPS 82 for *Bacillus thuringiensis* var. *israelensis* has been regularly checked on the test insect *Aedes aegypti.* A standardized assay is subject to limitations due to the difficulty of standardizing living organisms and to the way larvae react to the product. However, since 1982, the potency was considered stable with a coefficient of variation of less than 20%. Larval rearing was the most important factor in the reproducibility of the bioassay (Thiery and Hamon 1998).

It has been noted (Skovmand *et al.* 1998) that the calculation of the potency value of a product may be affected by many factors and especially by sample processing and pre-test rearing conditions in a laboratory. They propose that a standard test protocol should specify larval rearing conditions, such as type of food and feeding schedule, larval density, sample treatment methods, the size of bioassay cups, and the density of larvae in each cup. Samples should be homogenized to the single-cell level to reduce variation between laboratories and product types.

As an example, when laboratory bioassay studies were conducted on the efficacy of three commercial *Bti* products against 4 *Aedes* species, significantly different LC_{50} and LC_{95} values were determined (Brown et al. 2001). The authors conjectured that the differences in efficacy are related to formulation characteristics such as the number of particles on which the *Bti* toxins are carried per milligram. Furthermore, potency can be lost during formulation, processing, shipping and storage. The authors propose that mosquito control agencies evaluate the efficacy of commercial products against the local target *Aedes*. Products should then be selected using criteria including performance and not just price.

Assessments of *Bti* spores in water based on phenotypic (morphological structures) characters are insufficient when used alone in studies on the environmental ecology and fate of *Bt* because these methods do not provide unambiguous identification (Weir 2005). The Ministry of the Environment has developed procedures for the measurement of *Bti* in surface and ground water (Weir 2005). The analytical method includes three parts: (1) membrane filtration, (2) DNA extraction and purification, and (3) quantitative measurement of DNA specific to *Bti* with polymerase chain reaction (PCR) procedures. Measurement of *Bti* with this method can determine the presence/absence of specific endotoxin genes or whether a particular strain has lost or acquired specific δ-endotoxin genes in the environment. Naturally occurring *Bti* may contain between 1 and 4 of these toxin genes, while commercially applied *Bti* contains all four genes (i.e., Cry4Ba1, Cry11Aa1, CytAa1, and Cry4Aa1). Quantification is based on Real-Time PCR of the Cry4Ba1 gene only. Thus, counts of *Bti* as Cry4Ba1 more accurately reflect the amount of introduced or commercially applied *Bti* (Weir 2005). The detection limit for the MOE method was 58 spores per 100 mL (Weir 2005).

2.0 PRODUCTION PROCESS

2.1 Production

The production of *Bacillus thuringiensis* var. *israelensis* is described by Surgeoner and Fardas (1990). Only brief details of the processes are outlined below. *Bti* is produced in large fermentation vats. Three primary production suppliers for North America are: 1) Abbott

Laboratories in Chicago, Illinois, 2) Duphar laboratories in Belgium, and 3) Sandoz laboratories near San Francisco, California. Canada does not produce the active ingredient in *Bti* formulations (Surgeoner and Fardas 1990).

The details for the growth of bacterial growth and the media environment (i.e. pH, temperature, aeration level) are not available. However, the methodology of production for each company that grows bacteria commercially is similar to that for *Bti* (Surgeoner and Farkas 1990). The media for bacterial growth must contain carbon, nitrogen, and trace minerals. Sources of nitrogen may include fish meals, cotton seed flour, soybeans, autolyzed yeasts, and casein. Corn products, starch, and dextrose provide sources of carbohydrates along with trace minerals. The U.S. reference standard fermentation medium includes: generic tryptone (10 g/L), powdered corn (5 g/L), generic yeast extract (2 g/L, $K_2H\ PO_4$ and $KH_2\ PO_4$ each at 1 g/L) (Beegle *et al.* 1986).

Different *Bt* strains are inoculated into the steam-sterilized media in quantities ranging from 2 to 5% of the fermenter volume to initiate cell growth. The media is aerated and slowly agitated and pH is maintained between 7.2 and 7.6. Temperature of the media is regulated at approximately 30^0C (Surgeoner and Farkas 1990).

Vegetative growth of the bacteria occurs for five days (Surgeoner and Farkas 1990). In response to reduced nutrients in the medium, the bacteria develop into spores, each containing an endospore and protein crystals. The media is then heated to 100^0C for short periods to destroy the vegetative cells without harming the spores. After concentrating the endospores via

centrifugation or filtration, they are dried to form a powder. This powder is then bioassayed against the mosquito *Aedes aegypti* using accepted standard bioassay techniques. It is the concentrations of certain proteins making up the crystal and endospore coat that determine the potency of the *Bti* powder rather than the number of spores. In addition each fermentation process will produce a batch that will vary in potency, with an allowed variance of ± 20%, by international agreement. Different results with the same technical powder can occur with each bioassay (Surgeoner and Farkas 1990).

2.2 Commercial Products Containing *Bti*

Bti pest control products that are commercially available in Canada and are registered with the Pest Management Regulatory Agency include AquaBac XT, AquaBac II XT, AquaBac Granules, Vecto Bac 200G, Vecto Bac 600L, Vecto Bac 1200L, Gnatrol DG, Teknar Granules and Teknar HP-D.

Ontario's Pesticides Act, which is administered by the Ministry of the Environment, prohibits the sale and use of a pesticide product unless it is registered under the federal Pest Control Products Act (Ontario Pesticides Advisory Committee 1999). *Bti* pesticides are classified under Schedule 3 and are available for use to agriculturists, licensed exterminators, registered custom sprayers and the general public in accordance with allowed uses described on the product labels. Schedule 3 pesticides are considered minimally hazardous to human health and/or the environment if used according to recommended procedures.

Bti is available in a granular formulation (500 g shaker cans or 5 Kg bags) from local feed and hardware outlets, garden centres and pest control companies in Ontario. These vendors must hold a Pesticide Vendor's Licence. Rural landowners who have a pond or dugout that is contained on their property, with no outflows to other streams or lakes off their property, can apply specific products containing *Bti*. A farmer or rural dweller does not need a licence or permit to purchase or use a Schedule 3 product containing *Bti*.

Products currently registered, available in Canada, and classified for use in Ontario as Schedule 3 products include: AquaBac 200G Commercial PCP No. 26862, Vecto Bac 200G Commercial PCP No. 19466, and AquaBac 200G Domestic PCP No. 27374. Products sold in the United States, such as slow release dunks or pucks, are not registered in Canada.

One granular product is available for domestic use to control mosquitoes - AquaBac 200G. The AquaBac label states that an application of 100 granules of product per m^2 is equivalent to 0.5 mL of product per m^2 and would kill mosquito larvae within 24 hours after application.

Since, for water, 1 mL = 1 g, then the application rate can be restated as:

$$\frac{0.5\ \text{mL}}{\text{m}^2} = \frac{0.5\ \text{g}}{\text{m}^2}.$$

If the product remains on the surface of the water to a maximum depth of 0.1 m, then the concentration per m^2 of surface area is

$$\frac{0.5\ g}{0.1\ m^3} = \frac{5\ g}{m^3}$$

And since 1 m^3 = 1000 L, then the effective concentration is

$$\frac{5\ g}{m^3} = \frac{5g}{1000\ L} = 5\ ppm\ (mg/L)$$

2.3 Additives to Formulations

Initial products with *Bti* were crude powders of spores, crystals, growth media, and inert ingredients (Surgeoner and Farkas 1990). Formulations had suspendability problems, clogged spray systems, and provided inconsistent control. Today, *Bti* formulations are primarily spore and crystal concentrates prepared for use as water suspensions or oil emulsions. Additives have been incorporated into formulations such as thickening agents to provide uniform suspensions and wetting agents and sun screens to reduce the degradation of crystals by UV radiation. Concern has been expressed about the potential toxicity of these inert ingredients (Orton 1987). Product labels indicate the potency guarantee of the *Bti* but do not include concentrations of the other "inert" ingredients. These "inert" ingredients are critical to the success of new formulations and information about them is considered proprietary by companies. Because "inerts" are trade secrets, little public information is available about these chemicals. Swadener (1994) reviews the health problems that are available for the potentially toxic compounds which are used in *Bt* "inert" products.

A recent concern has been expressed regarding micro contaminants (USEPA-RED 1998). As shown by Fortin et al. (1986) the "inert" ingredients are perhaps the most toxic components of *Bti* formulations. Their toxicities would likely be minor when one considers the amounts applied in operational control programs according to studies done on "vehicle carriers" in the literature (Haverty 1982, Morris 1983). Proprietary toxicology data does exist on many of these standard "inerts" and on the formulated product. Federal agencies such as the Environmental Protection Service, Health and Welfare, and Fisheries and Ocean have reviewed this documentation. With permission of companies this information could likely be obtained from the companies or from the Pesticides Directorate in Ottawa (Surgeoner and Farkas 1990).

2.4 Quality Assurance

The U.S. Environmental Protection Agency (1998) requires a re-registration eligibility decision (RED) of the group of microbial pesticides registered as *Bacillus thuringiensis*. The decision includes a comprehensive reassessment of the required target data and the use patterns of currently registered products. *Bt* and strains of similar bacteria are used as insecticides on growing agricultural crops, harvested crops in storage, ornamentals, bodies of water, and around the home to control various groups of insects. EPA has concluded that all uses will not cause unreasonable risks to humans or environment and, as a result, all uses are eligible for re-registration. In addition to the toxins that are active against pests, *Bt* may produce undesirable toxins. To mitigate risks of potential toxicity to public and/or non-target species from these

toxins, the Agency is requiring continuation of the production batch quality control testing that originally appeared in the tolerance exemption and is requiring a re-evaluation and standardization of the manufacturing process for each registered technical grade of the active ingredient. In addition, several label changes are required of all *Bt* microbial products. The new product assessment will be updated because new genetic engineering techniques now allow genetic material encoding the delta-endotoxin insecticidal protein to be moved among subspecies to give different host spectrum ranges. Thus, EPA will no longer use the subspecies taxonomic unit as a primary differential characteristic of the species.

2.5 Formulation Potency and Molecular Structure

A crystal is a combination of proteins and a protoxin, (about 130-140 kDa) that has to be converted into a reactive form. This protoxin is highly insoluble in acidic (pH < 6.0) conditions and thus is safe to humans, higher animals and most insects (Deacon 2005; Knowles 1994; Li *et al.* 1991). At high pH (> pH 9.5), it is solubilized in the midgut of some lepidopteran (moths), dipteran (mosquitoes and black flies) and coleopteran (beetle) larvae.

Once the large (130-140 kDa) protein crystal dissolves in the alkaline midgut of the insect, the protoxin is split into an active delta-endotoxin of about 60kDa**.** This toxin binds to the midgut epithelial cells, which creates pores in the cell membrane and results in the equilibration of ions.

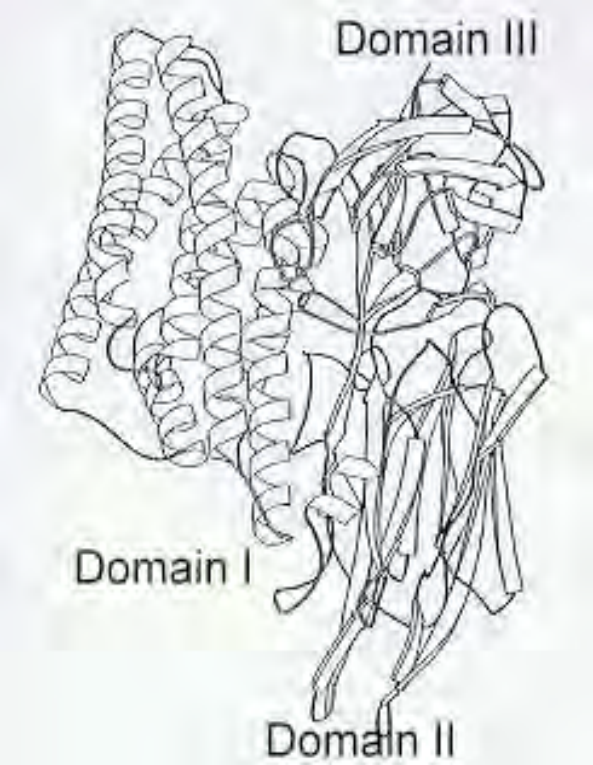

Fig. 1. Molecular structure of a delta endotoxin crystalline protein of *Bacillus thuringiensis*.

The gut pH is lowered by equilibration with the blood pH. As a result, the gut is immobilized, the epithelial cells break up and disintegrate, and the immature insect stops feeding. The lowered pH provides the means for the bacterial spores to germinate and causes an increase in vegetative bacterial cells in the host which, in turn, causes blood poisoning (septicaemia).

The delta-endotoxin has three domains (Fig. 1). Domain I is a bundle of 7 alpha-helices, many of which can penetrate the gut cell membrane creating a pore through which ions can pass freely (Li

et al. 1991; Prieto-Samsonov *et al.* 1997; Deacon 2005; Knowles 1994). Domain II consists of three anti-parallel beta-sheets which are thought to bind to receptors in the gut. Domain III consists of closely-packed beta-sheets which are squeezed together. This domain is believed to protect the exposed end (C– terminus) of the active toxin, thus preventing additional cleavage by gut proteases (Li *et al*. 1991; Deacon 2005; Knowles 1994).

In summary, at least four parameters are involved in the *Bti* crystalline protein's mode of action: 1) effectiveness of solubilization, 2) efficiency of protoxin-toxin conversion, 3) specific membrane receptor binding, and 4) membrane pore formation. The four factors determine the insect specificity of a crystal protein (Joung and Cote 2000).

2.6 Transgenic Expression of *Bti*

Current applications of *Bti* for mosquito control are limited by the short half-life of existing preparations under field conditions (Zaritsky 1995). To overcome this limitation, Xiaoqiang et al. (1997) and Lluisma et al. (2001) proposed cloning of the gene coding for the *Bti* in organisms inhabiting the breeding zones of mosquitoes and used by mosquitoes as a food source. A nitrogen fixing, blue-green alga (cyanobacteria) has been considered an attractive candidate for this purpose. An engineered organism carrying a combination of toxic genes when more than one gene is responsible for larvacidal activity would also reduce the concern that resistance might develop in mosquitoes. Algal clones of *Anabaena* (PCC7120) carrying cryIVA (cry 4A) plus cryIVD (cry11A) and p20 displayed toxicity against third-instar larvae of *Aedes aegypti*

(Xiaoqiang *et al.* 1997). Lluisma *et al*. (2001) reported evidence that *Anabaena* (PCC7120) strains expressing mosquitocidal toxin genes from *Bti* have a strong potential for biotechnological application.

3.0 TOXICITY

Bacillus thuringiensis is a gram-positive bacterium, widely used to control pest insects. During the last decade, *Bt*-based formulations have become more widespread, initiating the replacement of some harmful chemical pesticides. An extensive literature is available about the consequences of exposure of non-toxic organisms to *Bt* (WHO 1999) and the list of species that have been found to be susceptible to the toxic properties of *Bt* remains small.

The toxic properties are present in parasporal protein crystals. These protein inclusions constitute one to several types of δ-endotoxins [Cry (crystal) and Cyt (cytolytic) proteins]. Crystalline proteins have been reported (Prieto-Samsonov *et al.* 1997) to be toxic to different taxa within several invertebrate phyla: Arthropoda (Class:Insecta Orders:Lepidoptera-butterflies and moths; Diptera-some true flies; Coleoptera-beetles, Class: Arachnida-spiders, ticks and mites), Nematoda (segmented roundworms), Platyhelminthes (un-segmented flatworms, such as planarians, flukes, and tapeworms) and Protozoa (single-celled organisms like Amoebae and ciliates). The crystals are solubilized under the alkaline conditions of the invertebrate midgut and converted from protoxins into toxins with specific binding properties to different midgut receptor molecules

(Feldman *et al.* 1995, Rey *et al*. 1998). The bound endotoxins cause intestinal cell lysis and death by starvation or septicaemia. The Cry genes have been found in a large number of prokaryotic (cells lacking a nucleus and other membrane-bounded organelles, e.g. bacteria) and eukaryotic (cells with a distinct membrane-bound nucleus) organisms (Prieto-Samsonov *et al.* 1997). The toxicity studies summarized in this report are shown in Figures 2, 3, 4, 5 and Table 4.

3.1 Acute Toxicity: Non-target Aquatic Organisms

3.1.1 Vertebrates

3.1.1.1 Fish

Fortin et al. (1986) exposed brook trout (*Salvelinus fontinalis*) fry to *Bti* concentrations (product Teknar) of 4500 and 6000 mg/L and, within 45 minutes, 20% and 86.4% fry mortality occurred. However, no mortality was observed for fry exposed to 6000 mg/L of freeze-dried *Bti* in Teknar. At concentrations of 3000 mg/L and higher, fish fry became motionless and exhibited variable recovery within 2 h of exposure. Forty-eight hours of static exposure resulted in 1.33% mortality at 600 mg/L and no mortality at 300 mg/L. Because no mortality was observed with the freeze-dried Teknar product at concentrations of 6000 mg/L and because of the absence of effects in 48-h static tests, the authors concluded that the measured 2% xylene preservative agent in the Teknar formulation at the higher bioassay concentrations, instead of the exposure to *Bti*, caused mortality and changed the behaviour of fish.

Mortality of different life stages (eyed embryo to 82 mm fork length) of brook trout (*Salvelinus*

fontinalis), brown trout (*Salmo trutta*) and steelhead trout (*Oncorhynchus mykiss*) exposed to *Bti* in the laboratory increased when doses exceeded recommended label rates (Wipfli *et al.* 1994). The 48h-LC_{50} for brown and brook trout alevins ranged from 1561 to 2321 mg/L and were similar for both denatured (autoclaved) and nondenatured *Bti* tests, suggesting that mortality at these concentrations was due to additional proprietary chemicals in the formulation and not the *Bti* biocide.

Three-week-old fathead minnows, *Pimephales promelas*, were exposed to two commercial formulations of *Bti* in the laboratory. Larval mortality among fatheads exposed to 2.0×10^6 to 6.5×10^6 CFU/ml with both formulations occurred within 24 hours and resulted in 60% and 100% mortality, respectively, within 96 hours. Within 8 to 12 h of exposure, fish displayed signs of respiratory stress, i.e., gasping and swimming to the water surface. No mortality was seen at densities of 6.2×10^4 and 6.4×10^5 CFU/ml in 24 h. Dissolved oxygen in the test water declined from a mean of 7.0 mg/L in control and pre-dosed beakers to 6.4 mg/L in 6 h and to a low of 3.8 mg/L in 12 h in treatment containers. Dissolved oxygen in the controls remained stable during the entire experiment. The author concluded that mortality of larval fathead minnows was attributed to severe dissolved oxygen depletion due to formulation ingredients at high doses instead of direct toxicity from the *Bti* parasporal crystals (Snarski 1990). Another possible cause of stress in fathead minnows at high concentrations of *Bti* is that spores and crystals may be absorbed at gill surfaces and impede or block ion regulation.

Adult mummichog (*Fundulus heteroclitus*) fish were exposed to *Bti* and four other pesticides in

the laboratory (Lee and Scott 1989). The 96h-LC_{50} for the microbial larvacide *Bti* was 980 mg/L and was the least toxic to non-target fish of the six larvacides tested. The no observable effect concentration (NOEC) was 22.36 mg/L for *Bti* in Vecto Bac.

The larval stage of the guppy (*Poecilia reticulata*) was exposed to *Bti* in the laboratory (Mittal *et al.* 1994). The authors reported that this bio-insecticide was safe for the guppy based on 48h-LC_{50} >1000 mg/L. They concluded that this predatory fish could be used in conjunction with *Bti* to control pest mosquito larvae.

The mosquitofish (*Gambusia affinis*) has been shown to be an effective control agent for mosquitoes (Kramer *et al.* 1988). Addition of *Bti* at a concentration of 6 kg/ha together with mosquitofish at a density of 1.1 kg/ha was recommended as an effective mosquito control in commercially grown wild rice fields. *G. affinis* could tolerate *Bti* at this concentration without any apparent adverse effects.

3.1.1.2 Amphibians

No acute *Bti* studies on amphibians were found.

3.1.2 Invertebrates

3.1.2.1 Class: Crustacea

Toxicity tests of *Bti* and many other pesticides were conducted in the laboratory on the red swamp

crayfish, *Procambarus clarkii* (Holck and Meek 1987). Using different concentrations of *Bti*, an LC_{50} of 103.24 mg/L was determined. The authors concluded that the median lethal dose rates of *Bti* used in the crayfish bioassays were at least 500 times greater than the recommended maximum rates on the label for field applications to control mosquitoes. The high concentrations of *Bti* needed to produce an effect on crayfish may indicate a conclusion similar to that observed in the fish studies cited above in which formulation agents rather than the direct effects of the insect biocide *Bti* may be responsible for toxicity.

Static, acute toxicity tests with *Bti* granules and *Bti* liquid products were conducted on the crustaceans *Daphnia magna* and *Daphnia pulex* in moderately hard (U.S. EPA methods 1993) synthetic water (Milam *et al.* 2000). A 24h-LC_{50} of 626.6 mg/L was determined with *D. magna* using a *Bti* granulated formula. An exposure for 48 h with granulated and liquid formulae of *Bti* produced an LC_{50} of 0.34 mg/L and 0.0039 mg/L, respectively, for *D. pulex*. The authors concluded that the prevailing application rates of *Bti* and other pesticides for effective mosquito control can affect non-target organisms and may confound storm water and non-point toxicity evaluations that utilize sensitive indicator species.

3.1.2.2 Class: Insecta

A valid concern when dealing with an insect pathogen such as *Bti* is whether or not it will harm beneficial insects. From the literature available, it is evident that the use of *Bt* is safe for beneficial insects. A number of studies have dealt with this topic and several of these are

summarized here.

The fourth (final) instar of the aquatic midge, *Chironomus tepperi* (Family: Chironomidae), a major pest of rice, was used to determine the toxicity of *Bti* (Stevens *et al.* 2004). Tests were conducted using water dispersible granules of *Bti* (Vecto Bac WDG, 3000 ITU mg/L). Laboratory bioassays were conducted using different combinations of larval ages and densities to determine if these factors affected toxicity. Larval densities were 10 or 30 per container. Two stages of the fourth instar larvae were studied: young larvae at 6-7 days after culture establishment and older larvae at 10-14 days. The effects of temperature and substrate type were also studied. The two substrates consisted of river sand and autoclaved topsoil. The commercial product Vecto Bac was highly toxic to fourth instar larvae in bioassays using sand substrate with younger larvae being more susceptible (LC_{50} = 0.20 mg/L) than older larvae (LC_{50} = 0.46 mg/L). Increasing larval densities from 10 (younger: LC_{50} = 0.20 mg/L; older: LC_{50} = 0.046 mg/L) to 30 (younger: LC_{50} = 0.46 mg/L; older: LC_{50} = 0.080 mg/L) individuals per container increased toxic endpoints for both age groups. In addition, use of soil substrate increased the LC_{50} to 0.99 mg/L (older larvae, 10 per container) compared to river sand (LC_{50} = 0.46 mg/L). An inverse relationship with temperature was determined for LC_{50}'s using *Bti* WDG for older larvae with 10 per container in sand substrate. For example, at 30^0 C the LC_{50} was 0.19 mg/L and at 15^0 C the value was 1.88 mg/L. The authors concluded that water dispersible granules of *Bti* could effectively control *C. tepperi* at a field concentration of 2-3 mg/L in water 10 cm deep (Stevens *et al.* 2004).

The impacts of *Bti* were determined for non-target organisms utilizing bioassays and electron microscope studies (Yiallouros *et al*. 1999). Investigations were conducted on third- and fourth instar larvae of aquatic midges *Chironomus thummi thummi* (subfamily:Chironominae) and *Psectrocladius psilopterus* (subfamily: Orthocladiinae) to determine the sensitivity of species belonging to different subfamilies. The 24h-LC_{50} was 0.77 mg/L for *C. thummi* and 1.17 mg/L for *P. psilopterus*. The 48h-LC_{50} for the same two species were 0.33 mg/L and 0.57 mg/L, respectively. The authors reported that the mean 24 h toxic concentrations of *Bti* for these midge species ranged from 40-fold (*C. thummi*) to 60-fold (*P. psilopterus*) the LC_{50} for *Aedes aegypti* (0.019 mg/L) (Yiallouros *et al.* 1999). Ultrastructural investigations of the anterior mid-gut of midge larvae exposed to *Bti* showed cellular alterations similar to alterations in mosquitoes. This study showed that there is a difference in sensitivity to *Bti* for chironomid species in different subfamilies and that the susceptibility of chironomid larvae to *Bti* toxins is the result of internal damage of the midget epithelium that is similarly reported in target organisms (mosquitoes and black flies).

The potency of three different formulations of *Bti* (Bactimos, Vecto Bac and Teknar) was evaluated on the third and fourth instars of the midge larvae *Chironomus salinarius* (Diptera: Chironomidae). The 48h-LC_{50}'s for Bactimos, Vecto Bac and Teknar were 4.46, 5.4 and 14.63 mg/L, respectively. The authors concluded that, unlike organophosphate and pyrethroid insecticides, it was not economically feasible to use the *Bti* products as a biocide to control *C. salinarius* in local lagoons receiving large quantities of raw sewage (Ali *et al.* 1985).

Bioassays were conducted with mosquitoes and moth-flies that are commonly found in urban sewage environments, such as cesspools and septic tanks (Saitoh *et al.* 1996). The moth-fly is a non-biting fly (Diptera) that causes severe nuisance problems, such as the induction of allergic reactions in humans, and may carry sewage micro-organisms into indoor air environments. Several strains of *Bacillus thuringensis* found in Japan were assessed for toxic properties against mosquitoes and moth-flies. The results of the bioassays for the different of *Bti* were compared with a commercial strain of *Bti* that was used as a reference. The LC_{50} was 5.5 mg/L for *T. albipunctatus* and was far more toxic than other strains isolated from Japan. The authors concluded that the *Bti* strain and a few others would be good candidates to control sewage dipterans with just one biocide product.

The microbial insecticide *Bti* used to control mosquito larvae in Florida was tested on native non-target aquatic insects (Haag and Buckingham 1991). Laboratory bioassays were conducted on the water lily leafcutter moth *Synclita obliteralis* (Order: Lepidoptera; Family: Pyralidae), a common insect found living on aquatic plants in Florida. Fifty-three percent mortality was estimated for the caterpillar *S. obliteralis* at a *Bti* concentration of 0.020 mg/L. The amount of *Bti* required to affect the non-target aquatic moth is similar to the concentration that would be toxic to most mosquito larvae.

3.1.2.3 Other Insect Pest Species

Most of the studies to date using *Bti* have been on pest insects such as mosquitoes and black flies that are within the suborder Nematocera, Order: Diptera. However, pest flies within the suborder Brachycera: Family Tabanidae (horse fly and deer fly) have not been studied. Saraswathi and Ranganathan (1996) evaluated the efficacy of *Bti* against *Tabanus triceps* larvae (Tabanidae) and assessed which critical life stages are susceptible to this bacterium. Bioassays were carried out by exposing the 1st (1 hr old), 2nd (5 hr old), 3rd (2 day old), 4th (2 day old) and 5th (2 day old) instar larvae of *T. triceps* to different concentrations of *Bti.* It was determined that *Bti* was highly pathogenic to these larvae. The 24h-LC_{50} increased from first to fifth instar: 0.30, 0.36, 0.41, 0.47 and 0.52 mg/L, respectively. The toxicity of *Bti* decreased with increasing age and sensitivity was higher in the 1st instar than in the 5th instar. The 1st and 2nd instars of *T. triceps* are aquatic and mostly surface feeders similar to mosquitoes and would be easily affected by *Bti*. The later instars (3rd, 4th and 5th) of *T. triceps* live in water and slushy mud. The later instars get infected in two different ways: 1) from water during early times of treatment and 2) from soil during later times of treatment when *Bti* settles down on the bottom. Saraswathi and Ranganathan (1996) state that, although the later instars have a greater chance of contacting and ingesting the bacteria, their susceptibility is less due to food preference and feeding behaviour. Also, the midgut pH of the *T. triceps* larvae ranged from 8.8-10.5 and is similar to the gut pH of mosquitoes and black flies. In addition, larval midgut anatomy and physiology were determined by the authors to be similar to the gut of the latter two dipterans. Thus, *Bti* can be an effective control agent for tabanids (horse flies and deer flies).

The susceptibility of the biocide *Bti* was tested against the sandfly, *Phlebotomus papatasi*, a confirmed vector of cutaneous leishmaniasis in the Nile valley. Laboratory bioassays with this bacterium were conducted to measure both direct and indirect effects on immature and adult stages. The 32.5 day median lethal concentration for the larval stage of *P. papatasi* was 0.0026 mg/L. Normal development period for larvae was 29.5 days and was extended when exposed to *Bti*. The adult sand flies were fed a mixture of a sugary diet of fructose and *Bti* or glucose and *Bti* to produce 48h and 72h-LC_{50}'s of 13 mg/L and 3 mg/L, respectively. From their study of the effects of *Bti* on larval, pupal, and adult stages, the authors noted the extended effect of this bacterium not only by direct toxicity but also by altering the biology of adult life span which may interfere with the transmission of the Leishmania parasite (Wahba *et al.* 1999).

The effects of several formulations of *Bti* were evaluated against late instar black flies in bioassays using an orbital shaker that created water currents that simulated larval feeding similar to that in the natural environment (Barton et al. 1991). The most toxic formulation was 26-261-BD which produced a 5h-LC_{50} of 0.095 mg/L for a mixture of 80% *Simulium tuberosam* and 20% *S. notiale, S. verecundum, and S. aureum*. A 30-min exposure to *Bti* did not significantly alter the results. The authors concluded that the orbital shaker technique could greatly reduce the number of formulations applied in the field and save time, money, and other resources.

3.2 Chronic Toxicity: Non-Target Aquatic Organisms

3.2.1 Vertebrates

3.2.1.1 Fish

Populations of *Gambusia affinis* were exposed to *Bti* (Vecto Bac G 200) granules in experimental ponds from the end of June to early September at a maximum concentration of 6 kg/ha. No mortality was observed. In fact, populations of the mosquito fish increased by the end of the *Bti* exposure. In ponds with and without mosquito fish the *Bti* exposure significantly reduced the mosquito populations; however, the mosquito populations in ponds without fish rebounded to pre-treatment levels within two weeks. In ponds stocked with *G. affinis* and treated with *Bti,* mosquito populations remained low after *Bti* treatment. The authors concluded that mosquitoes in rice fields can be controlled with both *G. affinis* and *Bti*.

3.2.1.2 Amphibians

Only one study could be found on the effects of *Bti* on amphibians. In a 43-day growth study in which tadpoles were exposed to 10 mg/L of *Bti*, no mortality was observed for the frog *Rana temporara* (Paulov 1987). At concentrations of 10 mg/L, the growth (weight) of tadpoles was markedly delayed when compared with controls and metamorphosis was delayed by 4 days. At the end, all tadpoles transformed to the metamorphosis stage.

3.2.2 Invertebrates

No chronic toxicity studies were found on invertebrates.

3.2.3 Plants (Algae)

From previous studies Su and Mulla (1999) observed that, during mosquito control programs using microbial larvacides such as *Bti*, the productivity of some algae species was suppressed and that some water quality parameters were changed by primary productivity suppression. To test the validity of these algal observations, quantitative studies were conducted in microcosms where the mosquito species of *Culex* naturally breed (Su and Mulla 1999). In one treatment Vecto Bac G (200 ITU/mg) was added to the microcosms once at the beginning of the treatment at a concentration of 12.0 mg/L (48.1 kg/ha) and biotic and abiotic parameters were monitored for 28 days. The high doses of *Bti* yielded good control on days 2, 7, and 11 after treatment, with reductions of mosquito densities (*Culex spp*.) of 99, 88, and 59%, respectively (Su and Mulla 1999). The pre-treatment densities of the algae species examined in control and treatment containers were the same. On days 2, 7, 11, 16, and 21 post-treatment, the *Bti* treatment (12 mg/L) significantly ($p<0.05$) reduced the density of *Closterium* by 42, 48, 61, 97, and 95%, respectively. The pre-treatment densities of *Chlorella sp.* in control and treatment containers were the same. On days 2, 7, 11, 16, and 21 post-treatment, populations of *Chlorella* were

significantly reduced by 47, 81, 78, 99, and 95%, respectively, indicating *Bti* significantly suppressed the growth of this species (Su and Mulla 1999).

In addition to the suppression of algal growth with *Bti* addition (Vecto Bac G), differences were measured in dissolved oxygen concentration and water turbidity between control and treatment containers. With low algal productivity and the resultant reduction in photosynthesis with the high *Bti* treatment (12 mg/L or 48 kg/ha), oxygen concentrations were significantly ($p<0.05$) lower in the treatment than in the reference containers by 22, 39, 47, and 30% on days 7, 11, 16, and 21 after treatment, respectively (Su and Mulla 1999). Water turbidity was also different between reference and treatment containers. Turbidity values were significantly reduced ($p<0.05$) on days 2, 7, 11, 16, and 21 after treatment by 57, 62, 75, 86, and 80%, respectively.

In another treatment, *Bti* was added in the form of water dispersible granules (Vecto Bac WDG) at concentrations of 0.15 mg/L (0.6 kg/ha) for a 25-day period (Su and Mulla 1999). *Closterium sp.* and *Chlorella sp*. were present in approximately equal densities before treatment. The growth of *Closterium* in the treatment containers was significantly reduced on days 3, 7, 12, and 17 after treatment by 99, 98, 99 and 97%, respectively. For *Chlorella* the treatment significantly ($p< 0.05$) reduced the growth of this species on days 7, 12, and 17 by 98, 97, 95 %, respectively, compared to controls.

In addition to reduced densities of algae, significant reductions in oxygen measurements were 48,

41, 47 and 48 % on days 3, 7, 12, 17, respectively, after treatment. The turbidity values of water samples in the treatment tubs were significantly ($p<0.05$) reduced by 56, 77, 60 and 38%, respectively, on the same sampling days as for oxygen measurements.

The authors (Su and Mulla 1999) concluded that for Vecto Bac G (200 ITU/mg) the recommended dosage for the control of late instar mosquito larvae in polluted water with abundant algae is 11.4 – 22.7 kg/ha.

3.3 Acute Toxicity: Target Organisms

3.3.1 Mosquitoes

Mosquitoes are one of the groups of insects that are targeted for this pesticide and, therefore, are very sensitive to *Bacillus thuringiensis* var. *israelensis* (*Bti*) (Glare and O'Callaghan 1998; Joung and Cote 2000; Stark 2005).

Laboratory and field mosquito larvacidal efficacy of *Bti* depends upon a number of biotic and abiotic factors. Besides susceptibility differences of various mosquito species, factors such as larval stage, larval feeding behaviour, water temperature, water quality, light intensity, larval density and vegetative cover play an important role in the success or failure of the application.

A technical powder of *Bti* (Vecto Bac TP, 5000 ITU/mg), an aqueous suspension (Vecto Bac 12AS, 1200 ITU/mg) and a granular formula (Vecto Bac CG, 200 ITU/mg) were tested in the

laboratory and exposed to different physical and chemical conditions to evaluate the efficacy against larvae of freshwater (*Culex nigripalpus*) and saltwater (*Aedes taeniorhynchus*) mosquitoes (Nayar *et al.* 1999). Second, 3rd- and 4th-instar larvae of *C. nigripalpus* were 1.3- to 3-fold more susceptible to both Vecto Bac TP and Vecto Bac 12AS than were the larval instars of *A. taeniorhynchus.* For both species, the 2nd-instars were more susceptible to these *Bti* formulations than were the 4th instars. Larvae at lower densities (20 individuals) in test containers exposed to *Bti* showed 5-9-fold higher mortalities than larvae in containers at higher densities (50 and 100 individuals). Larval efficacy was maintained for both Vecto Bac TP and Vecto Bac 12AS suspensions for 24 hours. Larval efficacy was greater at 32-35^{0}C than at 15-20^{0}C. Significant loss of potency of both Vecto Bac 12AS and Vecto Bac TP was observed under 6 hours of high light intensity (140,000-170,000 lux) at 35-37^{0}C. A 50% increase in salinity levels compared to freshwater (0 salinity) caused a decline in efficacy of Vecto Bac 12AS and Vecto Bac TP against *A. taeniorhynchus* larvae. Vecto Bac GC caused insignificant initial and residual (up to 8 days) larval mortalities of both mosquito larvae. The GC formulation did not release the active ingredient of *Bti* in any significant mosquito larvacide concentration in a surface layer of water, and the formulation was more effective in shallow water.

Concentrations of *Bti* were tested in a bioassay system against fourth instar larvae of *Aedes aegypti* at different densities to reduce the deaths due to predation among test larvae (Misch *et al.* 1992). Such larval deaths are commonly encountered in bioassay groups of 25 larvae as currently specified in World Health Organization guidelines. The LC_{50} was affected by three factors: 1) number of larvae per container tested, 2) volume of the bioassay medium, and 3) length of time of

the bioassay. Mosquito larvae tested together in groups of 25 were twice (4h-LC_{50} = 0.03 mg/L) as sensitive to *Bti* as were larvae tested singly (LC_{50} = 0.06 mg/L). The authors concluded that predation and cannibalism were apparently the explanation for the free-floating head capsules that could frequently be found in grouped bioassays. It could also explain all of the deaths among controls as well as some inappropriately early deaths among larvae exposed to *Bti*. The volume produced another potential source of error, referred to as the threshold phenomenon. *Bti* tested against individual larvae for a period of 4 h in 1-ml volume resulted in an LC_{50} of 0.3 mg/L. *Bti* tested at the same concentration on single larvae in a 6-ml test volume resulted in a LC_{50} of only 0.6 mg/L. To explain this phenomenon, the authors suggested that the total amount of *Bti* in the 1-ml volume was insufficient to kill one larva during the experimental period, whereas the total *Bti* of 0.36 mg/L in the 6-ml volume test was sufficient to give an LC_{50} of 0.06 mg/L. In addition, lethality occurred at lower concentrations of *Bti* when tested over longer periods of exposure (single individual: 24h-LC_{50} = 0.021 mg/L vs. 48h-LC_{50} = 0.019 mg/L and groups of 25: 24h-LC_{50} = 0.011 mg/L vs. 48h-LC_{50} = 0.009 mg/L). Longer-term bioassays could increase the risk that feeding will decrease as moulting or pupation approaches. The smaller amount of *Bti* ingested could skew the results (Misch *et al.* 1992).

3.3.1.1 Effects of Temperature

Walker (1995) conducted experiments to determine the effects of *Bti* at low temperature (0^0C and 4^0C) and high temperature (22^0C) on the feeding rate and susceptibility of *Aedes stimulans*. Third-instar *A. stimulans* slowed feeding at 0^0C and 4^0C when compared to 22^0C. Susceptibility

of larvae to *Bti* was highest at 22^0C (LC_{50} = 0.1 mg/L) and lower at 4^0C (LC_{50} = 0.2 mg/L) and 0^0C (LC_{50} = 0.9 mg/L). The results of these experiments suggest that decreased efficacy of *Bti* at low temperature may occur because the rate of feeding decreases. Low temperatures should be a consideration during operational applications of *Bti* for control of larvae in cold-water habitats, such as the spring *Aedes* species (Walker 1995).

Christiansen *et al*. (2004) conducted laboratory bioassays on the salt-marsh mosquito *Ochlerotatus squamiger* to determine the efficacy of *Bti* (Vecto Bac TP) at different temperatures. Seventy-two hour bioassays on late 3rd- and early 4th-instar larvae resulted in LD_{50}'s of 0.23 mg/L at 6^0C, 0.12 mg/L at 10^0C, and 0.09 mg/L at 14^0C, respectively. Field trials corroborated laboratory bioassay observations producing 97-100% control of *O. squamiger* at 72 h post-application.

During the study of *Bti* potency, persistence, and dispersion in rivers, streams, and ponds, collecting a large number of samples is required and many cannot be processed immediately. Samples are usually frozen or kept at 4^0C to prevent or minimize enzymatic deterioration of crystals or bacterial growth before assaying. However, the potency of these *Bti* crystals when subjected to freeze-thawing cycles is not known. Tousignant *et al.* (1992) studied the effect of freezing and thawing *Bti* aqueous suspensions by looking at mortality response parameters such as the slope and LC_{50} of the probit regression. Initial concentrations of 1, 5, 10, and 20 mg/L at the moment of freezing of *Bti* suspensions did not significantly affect toxicity. However, the number of freeze-thaw cycles greatly increased the LC_{50}'s without much change in the slope of

the log-probit regressions. For example, for a single freeze-thaw cycle the LC_{50} was 0.11 mg/L and represents a 24% reduction in mortality (e.g., compared to an LC_{50} of 0.078 mg/L with no *Bti* freezing). Two freeze-thaw cycles produced an LC_{50} of 0.165 mg/L, while three cycles produced an LC_{50} of 0.237 mg/L. At the extremes, after 4 freeze-thaw cycles, the LC_{50} increased from 0.078 to 0.3 mg/L, indicating a 6.25-fold decrease in mortality. Thus, a concentration initially resulting in 50% mortality would result in less than 8% mortality after 4 freeze-thaw cycles (Tousignant *et al.* (1992).

3.3.1.2 Effects of Light

A granular formulation of *Bti* (Vecto Bac G - 200 ITU/mg) was found to be highly effective in reducing *Aedes aegypti* larval (4th instar) populations under laboratory conditions (Chui *et al.* 1995). The 24h-LC_{50} was 0.80 mg/L. Under continuous darkness the biological activity (determined by LC_{50} studies) of *Bti* was still quite effective after three weeks of storage, showing only a mild drop of 15.8%. Under continuous light, a large drop of 57.9% was determined, with insecticidal ability dropping rapidly after 4 days of storage. Under the photoperiod of 14:10 h (L : D), the decrease in efficacy was the same as under continuous light (55.6%). This light sensitivity accounted for the short residual effect of *Bti*.

3.3.1.3 Effect of Various Formulations

A truck-mounted ultra low volume (ULV) generator was used to disperse *Bti* (BMP 144 2X, Seleena *et al.* 1996). Mosquitoes were placed in containers at 3.3 m intervals, beginning with 3.3 m from the generator to 33.3 m in a straight line. Fogging with *Bti* continued for 30 minutes. Exposed and unexposed containers were then transported to the laboratory to determine mortality after 24 hours. The 24h-LC_{50} was 0.0027 mg/L. Complete larval mortalities for all the test mosquito species occurred within the first 23-27 m from the ULV generator. The authors concluded that the ULV method has the potential to disperse *Bti* to effectively control larvae.

Five organophosphates (OPs) (chlorpyrifos, chlorpyrifo methyl, fenthion, malathion, and temephos), three pyrethroids (bifenthrin, cypermethrin, and pemethrin), and two microbial pesticides (*Bti* and *Bacillus sphaericus* (*Bs*)) were tested as larvacides against the mosquito *Aedes albopictus* in laboratory experiments (Ali *et al.* 1995). The 24h-LC_{50}'s for Vecto Bac TP and Bactimos FC were 0.018 mg/L and 0.849 mg/L, respectively. Commercial products of *Bti* were considered economically effective against *A. albopictus* larvae but products of *Bs* were ineffective. In general, the toxicity ranking of the chemicals and microbials tested was: insect growth regulators (IGRs) > pyrethroids > Ops > microbials.

Table 3. Laboratory bioassays on different mosquito species (4th instar) with various formulations of *Bacillus thuringiensis* var. *israelensis*. Concentrations are in mg/L (Rettich 1983).

Species	Test	*Bti* Product			
		IPS-78	ABG-6108	R-153-78	Teknar
Aedes cantans					
	24h-LC_{50}	0.203	0.32	0.073	0.347
	48h-LC_{50}	0.157	0.22	0.064	0.229
A. communis					
	24h-LC_{50}	0.118	0.164	0.038	0.144
	48h-LC_{50}	0.074	0.088	0.02	0.071
A. punctor					
	24h-LC_{50}	0.2	0.203	0.069	0.228
	48h-LC_{50}	0.113	0.11	0.032	0.138
A. vexans					
	24h-LC_{50}	0.106	0.082	0.051	0.242
	48h-LC_{50}	0.088	0.054	0.03	0.136
A. cinereus					
	24h-LC_{50}	0.088	0.092	0.034	0.118
	48h-LC_{50}	0.07	0.053	0.018	0.068
Culex pipiens pipiens					
	24h-LC_{50}	0.155	0.123	0.08	0.09
	48h-LC_{50}	0.129	0.105	0.038	0.072
C. pipiens molestus					
	24h-LC_{50}	0.067	0.104	0.016	0.16
	48h-LC_{50}	0.051	0.08	0.015	0.105

The toxicity of four formulations (IPS-78, ABG-6108, R-153-78, and Teknar, Table 3) of *Bti* for six 4th instar species of mosquito larvae: *Aedes cantans*, *A. communis*, *A. punctor*, *A. vexans*, *A. cinereus*, and *Culex pipiens pipiens* captured in the field and one species from a laboratory strain (*C. pipiens molestus*) were tested (Rettich 1983). The author concluded that the *Bti* product R-

153-78 was markedly more effective than ABG-6108, Teknar or IPS-78. LC_{50}'s for the relatively large 4th instar species (*A. cantans*, *A. communis*, *A. punctor*) at 24 h were about 0.07 mg/L while for the smaller larvae of *A. vexans* and *A. cinereus*, *C. p. pipiens* and *C. p. molestus* the LC_{50}'s ranged 0.016 mg/L to 0.08 mg/L. Mortalities of larvae were observed within a few hours of exposure to *Bti*; however, significant differences in LC_{50} values usually occurred after 24-48 hours. The laboratory bioassay results were confirmed in field tests during which larval mortality continued up to 5-7 days after the spraying of *Bti*.

Two strains of *Bacillus thuringiensis* (*Bt* var. *israelensis* and *Bt* var. *kurstaki*) were assessed in the laboratory against different larval instars of the mosquitoes *A. aegypti* and *C. tarsalis* (Qadri *et al.* 1990). Both varieties of bacteria were found to be highly pathogenic for all age groups of the two mosquito species. Exposure to *Bti* produced 24h-LC_{50}'s of 0.06 mg/L and 0.15 mg/L (2nd instar), 0.1 mg/L and 0.17 mg/L (3rd instar), and 0.14 mg/L and 0.2 mg/L (4th instar) for the different ages of *A. aegypti* and *Culex tarsalis*, respectively. Larval bioassays for *Btk* produced higher LC_{50}'s for the same ages [LC_{50} = 0.15 mg/L and 0.27 mg/L (2nd instar); 0.2 mg/L and 0.5 mg/L (3rd instar); and 0.26 mg/L and 0.55 mg/L (4th instar)] and species (*A. aegypti* and *Culex tarsalis*), respectively. For both varieties of bacteria younger larvae of both mosquito species were more susceptible than older ones. Also, the rate of mortality was different for the two strains. For the variety of *Btk*, few larvae died immediately but they died slowly at a later time during the bioassay. However, larval death was immediate when exposed to *Bti*.

Laboratory bioassays were conducted on the toxicity of water-dispersible granules (Vecto Bac

WG, 3000 ITU/mg) of *Bti* on 3rd instars of six Australian mosquito species, *A. aegypti*, *O. vigilax*, *O. notoscriprtus*, *C. sitiens*, *C. annulirostris*, and *C. quinquefasciatus* (Russell et al. 2003). The 48h-LC_{50}'s for *C. annulirostris* and *C. quinquefasciatus* were 0.004 mg/L and 0.005 mg/L, respectively, and were greater than three times as susceptible as the most tolerant species (*C. sitiens*, 48h-LC_{50} = 0.019 mg/L). The LC_{50}'s were intermediate for *A. aegypti* (0.17 mg/L), *O. vigilax* (0.013 mg/L), and *O. notoscriprtus* (0.15 mg/L). Follow-up field studies were conducted on *C. annulirostris* using two formulations (Vecto Bac WG and Vecto Bac 12AS). Caged third instars were exposed to six concentrations of the WG formulation (0.004 – 0.13 mg/L) and three concentrations of the 12AS formulation (0.04-0.13 mg/L). Significant larval control (> 96%) 48 h post-treatment was produced at concentrations of 0.008 mg/L WG and 0.04 – 0.13 mg/L 12AS (Russell *et al.* 2003). Water quality was not affected by the treatment of the two formulations.

A new water-dispersible granular (WDG) formulation of *Bti* (Vecto Bac) was studied for the control of larval *Anopheles gambiae* mosquitoes in malaria-endemic Western Kenya (Fillinger *et al.* 2003). WDG and powder formulations were compared in laboratory bioassays. The *Bti* strains WDG and PP (powered formulations) showed high potency (LC_{50} = 0.021 mg/L and 0.006 mg/L, respectively) for *A. gambiae* under laboratory conditions. The authors concluded that the main malaria vector in their study area is highly susceptible to these microbial control agents. Minimum effective dosages to achieve elimination of the larval population in a given habitat are extremely low and environmental impact is negligible.

Zahiri *et al.* (2004) conducted laboratory bioassays of three microbial mosquito larvacidal

products consisting of *Bti*, *Bacillus sphaericus* (*Bs*) and a combination of *Bti* and *Bs* (University of California, UCR) recombinant against larvae of *Culex quinequefasciatus* (susceptible and resistant strains to *Bs*) and *Aedes aegypti*. *Bti* was highly effective against C. *quinequefasciatus* susceptible and resistant strains, with LC_{50}'s of 0.009 mg/L and 0.011 mg/L, respectively. *Bs* was highly active against the susceptible strain of *C. quinequefasciatus* with an LC_{50} of 0.006 mg/L. *Bs* exhibited little toxicity against *A. aegypti* and almost no toxicity to *Bs* resistant strains. The UCR recombinant was equally active against both *Bs*-susceptible and -resistant strains of *C. quinequefasciatus*. LC_{50}'s were 0.005 mg/L and 0.009 mg/L, respectively. *Bti* and the UCR recombinant showed similar activity for both *Bs*-susceptible and -resistant strains. UCR recombinant showed high toxicity to *A. aegypti* with an LC_{50} of 0.023 mg/L. In the field, *Bti* and low-concentrate UCR recombinant showed similar initial activity as well as persistence. At low doses, residual activity of *Bti* and UCR recombinant lasted for < 7 days. *Bs* and high-concentrate UCR recombinant were more effective against natural populations of *Culex* and achieved longer control (7-21 d) than did the other two products (Nayar *et al*. 2004).

A formulation of *Bti* (Teknar HP-D) was tested in laboratory experiments against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (Gunasegaram *et al.* 2004). The toxicity of *Bti* to a mosquito fish predator, *Gambusia affinis* and to the water bug predators, *Notonecta* sp. and *Diplonychus indicus* were also evaluated in the laboratory. The LC_{50}'s for *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* exposed to *Bti* were 0.00174 mg/L , 0.00213 mg/L and 0.00277 mg/L, respectively, with *A. aegypti* being the most sensitive. The fish *G. affinis* which was exposed to a dose ranging from 0.032 mg/L (1.0 L/ha) to a maximum of 3.2 mg/L (100 L/ha)

showed no mortality after a test period of 5 days. In addition, no mortality of the two water bugs *Notonecta* and *D. indicus* was observed after 2 days predation on the surviving mosquito larvae of *C. quinquefasciatus* subjected to sub-lethal dosages of *Bti* (Teknar). The authors concluded from the data that *Bti* is safe to non-target organisms.

Toxicity of two larvacidal formulations of *Bti* (Vecto Bac and BMP-144-2X) was determined against *Anopheles stephensi* and *Aedes aegypti*, the two major urban mosquito vectors of malaria and dengue fever (Mittal *et al.* 2001). The study showed that the two formulations of *Bti* were highly toxic against both mosquito species. Of the two *Bti* formulations, Vecto Bac was more toxic than BMP-144-2X for both species. The LC_{50}'s for *A. aegypti* and *A. stephensi* were 0.064 and 0.14 mg/L, respectively, for Vecto Bac and 0.145 and 0.81 mg/L, respectively, for BMP-144-2X.

Laboratory studies were conducted to test *Bti* (IPS-82) on the 2nd and 3rd instars of the mosquito *Anopholes arabiensis* (Seyoum and Abate 1997). The 48h-LC_{50} was 0.001 mg/L for the 2nd and 0.0018 mg/L for the 3rd larval stage. Thus, half the toxic crystalline proteins are needed in the same volume of water to cause mortality of the second instar than the third instar of *A. arabiensis*. The authors concluded that the larvacidal potential of *Bti* is very promising as a vector control for malaria.

The susceptibility of field collected *Aedes aegypti* larvae was evaluated in terms of median lethal time (LT_{50}) and final mortality after exposure to *Bti* (de Andrande and Modolo 1999). The

median lethal time was determined for two potencies of *Bti*, 2500 ITU/L and 5000 ITU/L for 3rd and 4th larval instars. For 2500 ITU/L of *Bti* the median lethal time for *A. aegypti* was 261.2 minutes for the 3rd instar and 391.4 minutes for the early 4th instar larvae. For 5000 ITU/L of *Bti*, the median lethal time was 235 minutes for the 3rd instar, 298 minutes for the early 4th instar and 362 minutes for the late 4th instar. Final mortality after 18 h of exposure to *Bti* was 100% for the two larval instars. Thus, the third instar larvae were shown to be more susceptible than early and late fourth instar stages (de Andrande and Modolo 1999). The LT_{50}'s of *A. aegypti* ranged between 4 to 6.6 h when subjected to recommended field doses.

3.4 Chronic Toxicity - Target Organisms

3.4.1 Mosquitoes

Laboratory bioassays were conducted to determine the efficacy of *Bti* (Vecto Bac TP, technical powder and Vecto Bac WDG, water dispersal granules) at different concentrations and temperatures on the salt-marsh mosquito, *Ochlerotatus squamiger* (Christiansen *et al*. 2004). Seventy-two hour bioassays produced different LC_{50}'s at different temperatures in the laboratory. At temperatures of 6^0C, 10^0C, and 14^0C, the 72h-LC_{50}'s for *Bt*i (Vecto Bac TP) were 0.226 mg/L, 0.112 mg/L, and 0.09 mg/L, respectively. The product Vecto Bac WDG was not as effective, giving an LC_{50} of 0.147 mg/L at 10^0C.

3.5 Summary of Toxicity Data to Aquatic Organisms

3.5.1 Acute

Acute toxicity estimates for fish exposed to *Bti* were between 980 and 10,000 mg/L (Fig. 2; Table 4). Although a concentration of 1.0 mg/L of *Bti* was reported for *Gambusia affinis* for a 24 h bioassay, no mortality was measured at that concentration (Milam *et al.* 2000). In most studies on fish, it was concluded that mortality was due to formulation products (such as high xylene) or indirectly to reduced oxygen levels resulting from very high concentrations of *Bti* in bioassays instead of the direct toxicity from *Bti* endospores.

For invertebrates, acute toxicity values reported for non-target organisms ranged from 0.0026 mg/L to 626.6 mg/L (Fig. 2; Table 4). The most sensitive organisms were the sandfly, *P. paptasi* (Wahba *et al.* 1999), the water flea, *D. pulex* (Milan et al. 2000), and the aquatic caterpillar, *S. obliteralis* (Haag and Buckingham 1991). For target organisms, acute toxicity concentrations ranged from 0.001 mg/L to 0.849 mg/L. The mosquito, *Anopheles arabiensis* (Seyoum and Abate 1997) was the most sensitive organism to acute exposure to *Bti.*

3.5.2 Chronic

There were no chronic studies using *Bti* that were considered primary data. *Bti* exposure for 43 days reduced the growth of the non-target amphibian, *Rana temporaria*, by 10% (Paulov 1987);

however, no mortality was reported (Fig. 2; Table 4).

One 72 h chronic study was conducted on the target salt-marsh mosquito *Ochlerotatus squamiger* exposed to two *Bti* products: Vecto Bac WDG (water dispersal granules) and Vecto Bac TP (technical powder) at 10^0C. The LC_{50}'s were 0.147 mg/L and 0.122 mg/L, respectively (Fig. 2; Table 4).

3.6 Aquatic Ecosystem Effects

Most of the studies cited in this review (primarily laboratory studies (Table 4 and Figures 2, 3 and 4) relate to direct (toxicity) short-term effects of *Bti* application to control mosquitoes in aquatic ecosystems. Routine treatment of mosquitoes with *Bti* in a variety of aquatic habitats is on the increase throughout the world (Table 4). However, relatively few studies have evaluated the long term impact of this bacterium in ecosystems (Lacey and Siegel 2000). Compared to the target blackfly species in flowing water ecosystems, a paucity of studies exists on the effects of *Bti* applications on overall ecosystem community structure and function in lentic ecosystems inhabited by mosquitoes (Bousvert and Bousvert 2000). In addition, relatively few long-term studies exist, either with single and/or multiple applications of *Bti,* in which both direct and indirect toxic effects have been studied.

A notable exception was done on Minnesota wetlands. Hershey et al. (1998) conducted a long-term study on the effects of multiple *Bti* applications on non-target organisms. This study was

conducted over a five-year period. The first two years consisted of pre-treatment and reference sampling of multiple wetlands without applying *Bti*. Subsequently, *Bti* additions occurred for three consecutive years. *Bti* was applied six times each year between the months of mid-April to mid-July at rates recommended on the label. However, although rates of *Bti* were added to achieve a desired concentration according to label instructions, six times per season would be considered excessive for mosquito control (Bousvert and Bousvert 2000). Little change was measured in community structure after the first year of *Bti* application. Significant changes were measured in some insect taxa during the second year. During the third year many insect taxa were depleted in the treated wetlands relative to reference areas. Hershey *et al.* (1988) reported dramatic changes in diversity indices in benthic (bottom-dwelling) organisms with a drastic reduction in richness while dominance of a few groups increased. The large reduction in benthic community structure as a result of repeated exposures to *Bti* (six times per season at 3-week intervals) was thought to influence reductions in non-target organisms through both direct and indirect effects. The authors (Hershey *et al.* 1988) hypothesized that the repeated treatments of *Bti* had long-term impacts on wetland non-target communities by disruption of the invertebrate food web.

In a separate study Su and Mulla (1999) added *Bti* to control mosquitoes and also provided results on the indirect effects of these *Bti* applications on changes to phytoplankton structure and function. These authors added two *Bti*-coated granule formulations (Vecto Bac G and Vecto Bac WDG) in different experiments to assess controlling *Culex* mosquitoes in microcosms. Su and Mulla (1999) reported significant reductions in growth in the same experiments for two species of

green algae (*Closterium, Chlorella*) for up to three weeks. These authors concluded that in addition to the reduction of mosquito larvae there was an associated decrease in primary production due to reduced algal growth.

3.6.1 Water Depth

Another important factor that could alter the toxicity of Bti to target organisms is water depth. The amount of *Bti* product added to mosquito habitats is based on the surface area of water to be treated. Thus the final potency (ITU/mg) and concentration (mg/L) can be quite variable depending on water depth (see Table 1). Charbonneau et al. (1994) studied the influence of depth on Vecto Bac G efficacy in a Minnesota wetland. They reported that the effect of Vecto Bac G on the midge larvae *Chironomus riparius* was inversely related to water depth. For example, at depths ranging from 6.7 to 9.1 cm there was 100% mortality when exposed to *Bti* at the recommended application rate (5.6 kg/ha), whereas mortality varied from 0 to 25% in 40.6 cm deep water.

Russel *et al*. (2003) stated that it is difficult to directly compare *Bti* field results at measured depths in their experiment with all other publications on Vecto Bac products because application rates are expressed in so many different ways and at different depths. For example, Ragoonanansingh *et al*. (1992) and Fallinger *et al*. (2003) calculated product concentrations assuming a water depth of 10 cm. The study ponds were actually deeper, underestimating product concentration. The long-term wetland study in Minnesota (Hershey *et al.* 1995; 1998; Neimi *et*

al. 1999) had variable wetland water levels within a year and over several years of constant multiple *Bti* application rates, all based on surface area. Some of the variable invertebrate toxicity results among years could be attributed to variable water depth. Russel *et al*. (2003) and Christiansen *et al.* (2004) state that, when selecting an appropriate application rate for mosquito habitat, the depth of the water should be considered. Measuring the depth, then calculating the amount of *Bti* required, provides the best indication of product concentration that is dispersed throughout the water column.

4.0 MUTAGENICITY

The potential exists for mutagenic changes in *Bti* because it is a living organism (Surgeoner and Farkas 1990). Over 31 recognized subtypes (DSMZ 1994) and 800 strains (Dulmage and Cooperators 1981) indicate that genetic variability exists. Genetic engineering techniques now allow genetic material encoding the delta-endotoxin insecticidal protein to be moved among bacterial subspecies and to other plants and animals (Prieto-Samsonov *et al*. 1997). Therefore, the U.S. EPA will not longer use the subspecies taxonomic unit as a primary differential characteristic of the species. EPA will consider each new strain (a pure culture of descendents of a single isolation) of *Bt* as a new active ingredient (U.S.EPA-RED 1998).

Pathogenic mutations of *Bacillus thuringiensis* are rare since natural epizootics do not occur in nature or in years following spray programs. *Bt* strains appear to be specially adapted to insects

and poorly competitive against other microbes. The lack of widespread natural epizootics would indicate that it is genetically a relatively stable organism with little chance of non-insect infection (Surgeoner and Farkas 1990).

The US Environmental Protection Agency (2005) has not identified any sub-chronic, immune, endocrine, or non-dietary cumulative exposure issues that may affect children and infants or the general population after 30 years of widespread *Bti* use. In addition, no confirmed reports of delayed or immediate allergic reactions to the delta-endotoxin have been reported even though there have been many studies of oral, dermal, and inhalation exposure to *Bti.*

Because *Bti* is a live organism, testing for hazards of this bacterium is done differently than for conventional chemical pesticides (Swadener 1994). The US Environmental Protection Agency does not require studies to determine mutagenicity, carcinogenicity, or chronic toxicity. Controlled laboratory tests are conducted to determine the ability of microbial pesticides to cause disease (pathogenicity) and reproduce within the host's body (infectivity).

In studies conducted on rodents, 79% mortality occurred in rats when a single high dose of *Bti* was injected into the brain (Siegal and Shadduck 1988). Spleens became enlarged in mice injected with *Bti* (Siegal and Shadduck 1990). *Bti* exposure to eyes of rabbits produced irritation with the severity depending on the size and compactness of *Bti* spore particles (Siegal and Shadduck 1988).

5.0 CARCINOGENICITY

No oncogenicity studies of *Bti* effects on non-target organisms were found in the literature. No tumors were observed in 2-year chronic studies where rats were given dietary doses of 8400 mg/kg/day of *Bacillus thuringiensis* formulation (Abbott 1982).

6.0 BIOACCUMULATION

Laboratory studies by Snarski (1990) on the interactions between *Bti* and fathead minnows (*Pimephales promelas*) showed that bioaccumulation of this biocide in fish is of short duration. *P. promelas* minnows accumulated whole-body counts of 4.0×10^6 CFU (colony forming units) in each fish when exposed to 2.2×10^5 CFU/mL of *Bti* for one hour. Ingestion was determined to be the major route of exposure. The spore counts of *Bti* decreased rapidly by 3 orders of magnitude within 24 h after transfer of fish to clean water. Detection of spores was at a minimum in fish after 8 days but low numbers were measured in feces for approximately 2 weeks. The author concluded that *P. promelas* could enhance the dissemination of *Bti* spores in the aquatic environment via ingestion and excretion.

7.0 ODOUR AND TASTE

No data were found concerning the taste or odour effects of *Bti* on the quality of the water.

8.0 DERIVATION OF AN ONTARIO PROVINCIAL WATER QUALITY OBJECTIVE

For purposes of criteria development, toxicity data are classified by OMOE as primary or secondary, acute or chronic. All candidate toxicological information is screened for acceptability (Table 4, OMOE 1992). To be considered primary, the following must apply: accepted laboratory practices of exposure and environmental controls must be followed; stable, measured concentrations of toxicant throughout the test must be maintained; test endpoints and lengths of exposure appropriate to the life stage of the species tested and the characteristics of the substances must be used; relevant environmental parameters such as temperature, pH and hardness must have been recorded; responses and survival of controls must be appropriate for the test species and test used. Data on vertebrates and invertebrates not meeting all of the above are denoted as secondary in objective development documents.

The terms acute and chronic in connection with toxicity tests refer to short (acute) and long (chronic) exposure. Such terms require a specific organism as a reference. For a *Daphnia* with a life cycle of weeks, acute usually means two days or less. When classifying test results for a substance as either acute or chronic based on the duration of exposure, the nature of the substance and how rapidly it acts is considered. More details are given in OMOE (1992).

The minimum database required to develop a numeric Provincial Water Quality Objective was not met for *Bacillus thuringiensis* var. *israelensis*. Boisvert and Boisvert (2000) reported that the

effects of *Bti* on non-target organisms are hard to predict due to 1) differences in the species evaluated, 2) different methodologies used in the laboratory and in the field and 3) different formulations used in the research studies. For the studies reviewed in this report the chemical concentrations of the bioassay test water, *Bti* spore densities and crystalline endotoxin densities within the spores during the bioassays (Table 4) were rarely measured. Variable spore counts (Colony Forming Units, CFU) were originally used in earlier studies as a way to measure toxicity (Snarsky 1990, Boisvert and Boisvert 2000). However, Dulmage (1973) reported no correlation between toxicity and spore count. The crystalline endotoxins in the bacterial spores are the toxic agents. The toxic activity is determined by assessing the potency (expressed as International Toxic Units/ mg) of the reference formulation using a standard organism (i.e., *Aedes aegypti*) and comparing it to the commercial formulations. Mulla (1990), however, found no consistent relationship between potency (ITU/mg) and toxicity to mosquito larvae. In addition, the formulations and potencies used in the summary toxicity studies in this report (Tables 2 and 4) were variable. A method now exists to measure spore densities in water (Weir 2005), but the link between *Bti* spore densities, protoxins and endotoxin crystals, of which the latter ultimately kills the mosquitoes and other nematocerans, has not been studied.

Therefore, based on the difficulty of correlating enumerated bacterial spores with potential toxicity to target and non-target aquatic organisms, development of a numeric *i*PWQO is difficult. Instead a narrative value is proposed in which application of *Bti* at ~ 1.0 mg/L will result in a small, if any, impact on aquatic ecosystems. However, most mosquito larvae will be impacted at the 1.0 mg/L concentration. Some non-target chironomids (midges) are sensitive to *Bti* at higher

concentrations than most mosquito species at recommended label concentrations of 5 mg/L (Fig. 2, Table 4). For example, in laboratory experiments Charbonneau *et al.* (1994) reported that several genera of chironomid larvae were susceptible to Vecto Bac G at levels substantially lower than those recommended for field application. However, all subsequent field experiments indicated that Vecto Bac G did not reduce abundances of the benthic community, including chironomid midges when applied at the operational level or even at five times the operational level.

It is recommended that not more than one to three applications of *Bti* be applied per year. Hershey *et al.* (1988) documented significant reductions in benthic insect communities in addition to mosquitoes with *Bti* application rates of six times per year over multiple years.

9.0 A NARRATIVE OBJECTIVE VALUE

The proposed *i*Provincial Water Quality Objective for target mosquitoes is the application of *Bti* at concentrations (~ 1.0 mg/L) below the recommended label concentrations (operational doses).

This application at below the operational concentration will protect most species at all life stages for their entire life cycle.

10.0 RESEARCH NEEDS

One long-term study in wetlands has shown significant dramatic effects from repeated doses of *Bti* treatments on non-toxic organisms. More long-term studies are required to determine if yearly doses of *Bti* will significantly alter aquatic food webs. In addition, more chronic laboratory studies are needed on non-target invertebrates and algae. There is some limited information that green algae may be drastically impacted by *Bti* exposure. Also, more research is needed concerning the link between *Bti* spore densities, protoxins and endotoxin crystals, of which the latter ultimately kills the mosquitoes.

11.0 SUMMARY

For non-target organisms bioassays using *Bti* were conducted on 7 fish species and 16 invertebrate species (Fig. 2, Table 4). The duration of the fish studies ranged from 3 h to 96 h. A bioassay on an amphibian species was conducted for 43 days. The duration of the bioassays for invertebrates ranged from 5 h to 96 h. Two genera of microscopic plants (algae) were studied over two time periods of 2-3 days and 7 days.

Of all the non-invertebrate species evaluated for *Bti* toxicity, 3 species (sand fly, black fly and

horse fly) are known as pests because they seek blood meals from humans. Out of the 16 studies on invertebrates, 63% had toxicity values below the recommended application rate (AquaBac, 5 mg/L) for the domestic commercial product. AquaBac 200G has one of the lowest toxicity potency ratings of all the products (Table 4) used in the toxicity studies cited in this report.

For target species (Figures 3 and 4, Table 4) approximately 87 bioassays were assessed on 30 species of mosquitoes from a wide range of countries using 19 different *Bti* products. All but 5 studies were done on freshwater species. Five salt water species were included in this report because their habitats and distributions could be influenced by the significant use of road salt in the Province of Ontario.

For target organisms the maximum 48h LC_{50} (Fig. 4) was 0.31 mg/L for the 4th instar of *Ae. atropalpus* with a mean LC_{50} of 0.094 mg/L (standard deviation = 0.067, n = 32) for all 4th instar species and all bioassays. Mean 48h LC_{50}'s for 2nd and 3rd instars were 0.0047 mg/L (S.D. = 0.003, n = 4) and 0.0249 mg/L (0, 034, n = 7), respectively, for all species. The maximum 24 h LC_{50} was 0.85 mg/L for the 4th instar of *Ae. albopictus.* Mean LC_{50}'s for 2nd, 3rd, and 4th instars were 0.043 mg/L (S.D. = 0.057, n = 6), 0.129 mg/L (S.D. = 0.17, n = 26) and 0.187 mg/L (S.D. = 0.31, n = 12), respectively, for target mosquitoes.

The potency of the *Bti* products used in the 19 different products ranged from 200 to 15,000 ITU/mg (Table 2 and 4). Approximately 50% of the bioassays were conducted for 24 h (Fig. 3) and 50% for 48 h (Fig. 4). In 80-90% of all bioassays the tests used 3rd and 4th instar larvae.

Second instars were used in the other 10-15% of the bioassays. Based on all the data, the genera *Culex* and *Ochelrotatus* are more sensitive to *Bti* than *Aedes* and *Anopheles*. More specifically, *Aedes* and *Anopheles* had mean effective concentrations of 0.122 (S.D. = 0.165, n = 52) and 0.129 (0.263, 9), respectively. The mean effective concentrations of *Culex* and *Ochlerotatus* were 0.061 (0.077, 21) and 0.066 (0.065, 4), respectively.

A summary of the ranges (Figure 5) of non-target and target toxicity data, regardless of the large range in product active ingredients (i.e., ITU/mg) and variable bioassay water quality (Table 4 and Figs. 2-4), reveal that the recommended operational dose of *Bti* products of 5 mg/L or greater to control mosquitoes in surface waters may be too high. Sixteen toxicity tests (47%, Figure 5) of a total of 34 (Figure 2) showed that 14 non-target species (10 vertebrate and 4 invertebrate species) out of 23 (61 %) produced LC_{50} values of 1.0 mg/L or less. All of the non-target vertebrates (7 species) and 4 of the invertebrate species tested are considered not sensitive and were well above 5 mg/L. Thus, a more appropriate concentration of *Bti* to add to water is ~ 1.0 mg/L. This 1.0 mg/L concentration of *Bti* would kill most of the target mosquito species while protecting more of the non-target species.

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Figure 2 *Bti* Derivation Graph - Non-Target Organisms

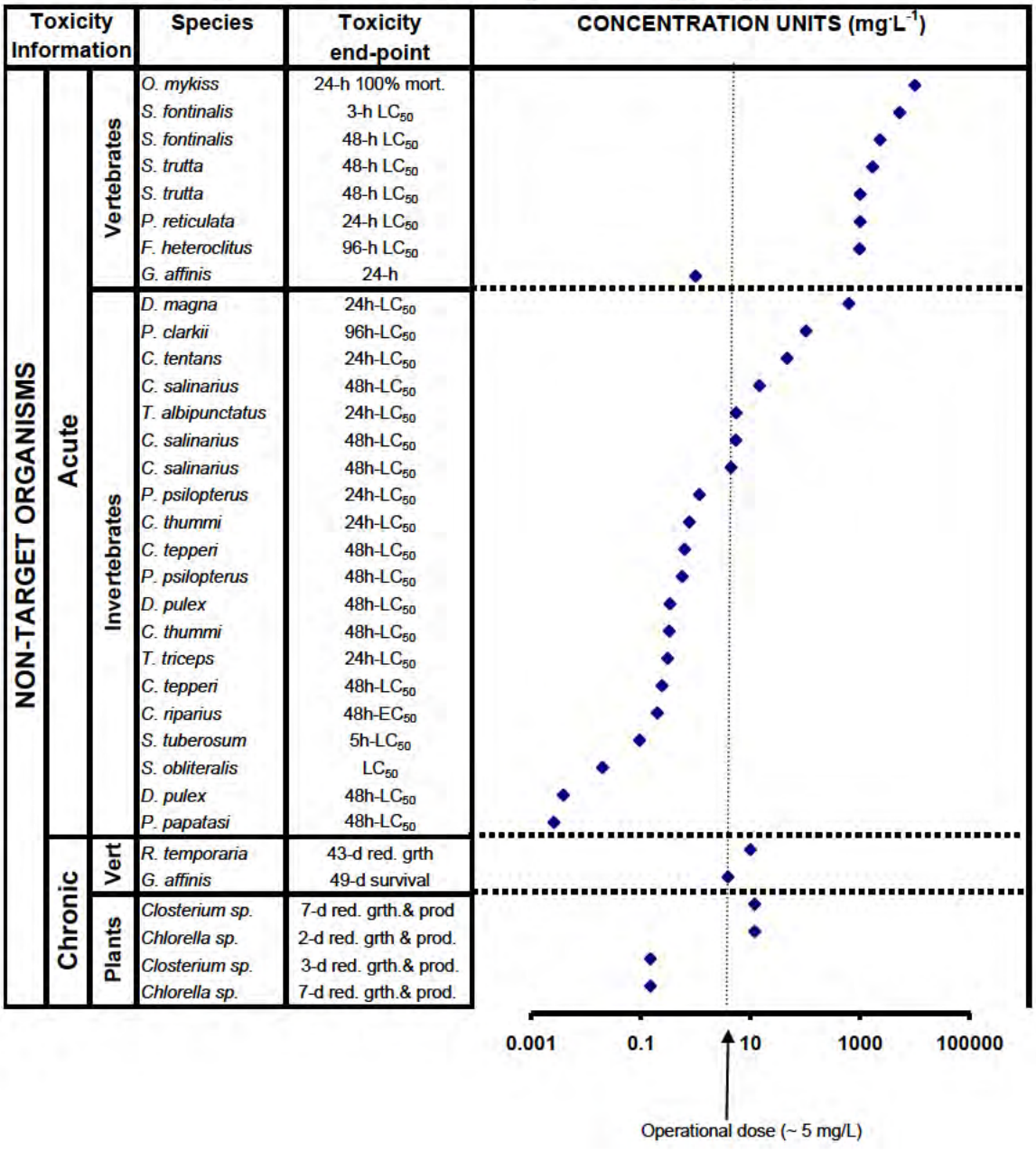

Figure 3 *Bti* Derivation Graph - Target Organisms

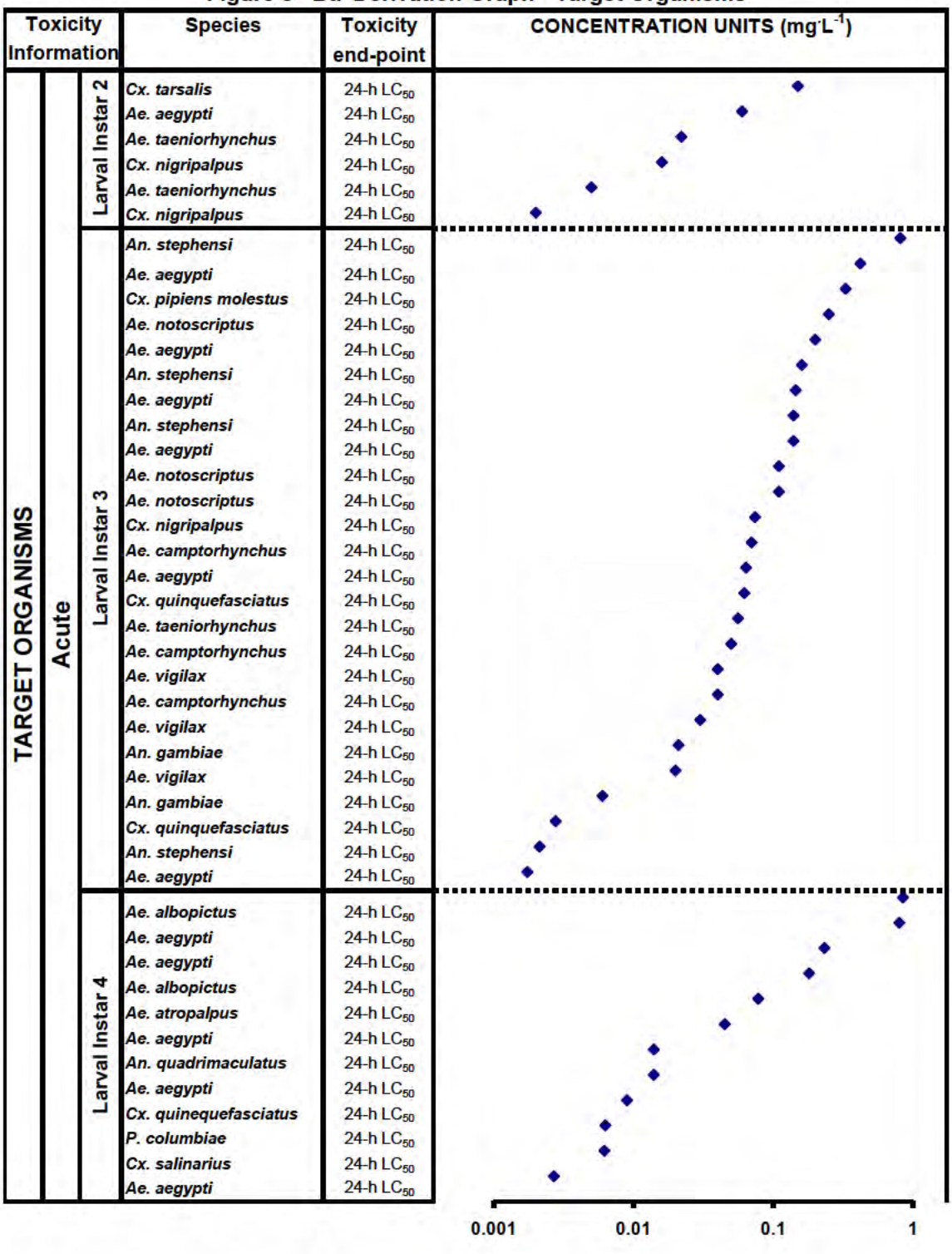

Figure 4 *Bti* Derivation Graph - Target Organisms

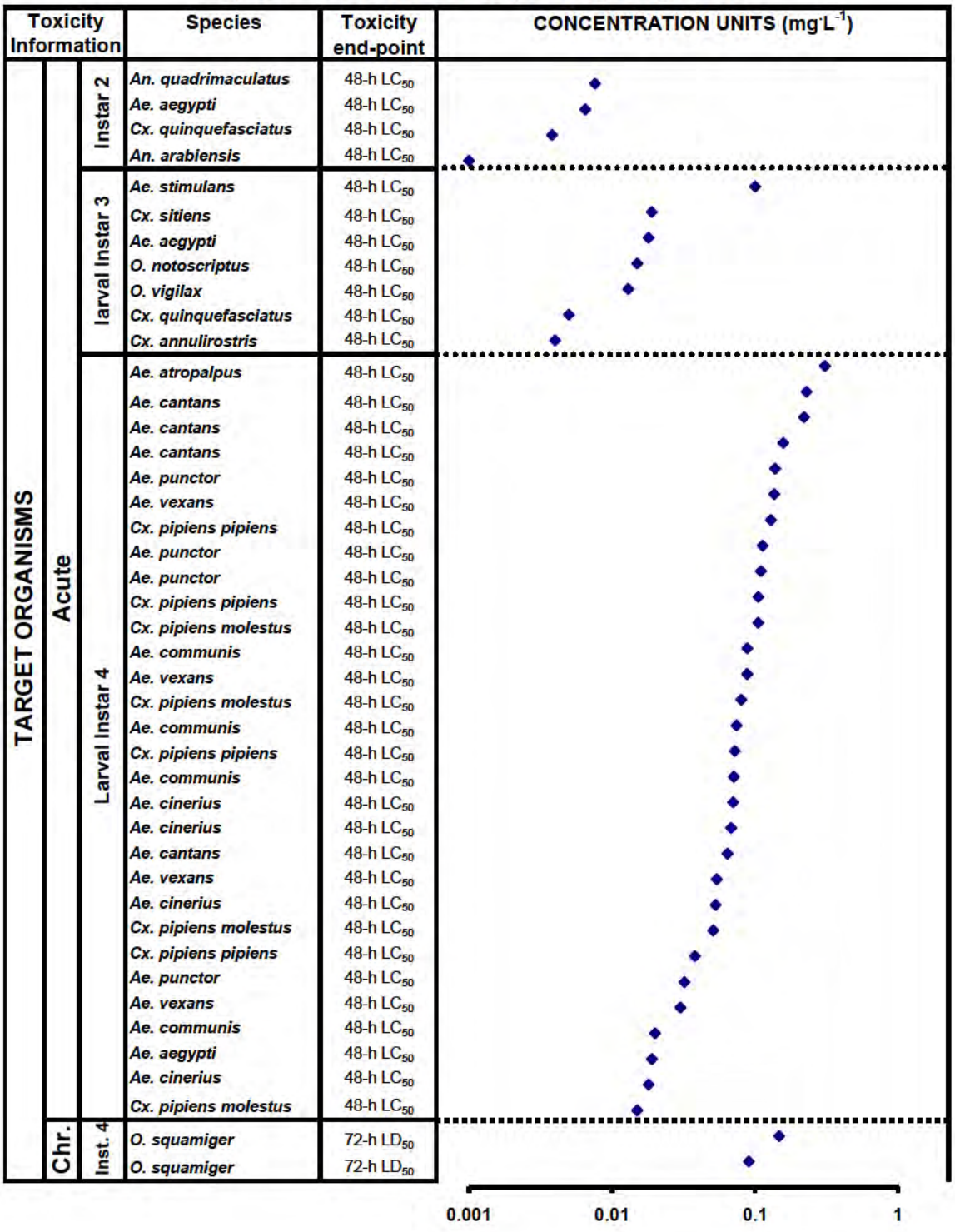

Figure 5. **Range (log_{10} concentration) of *Bti* toxicity endpoints (LC_{50}-mg/L) for non-target and target aquatic biota.**

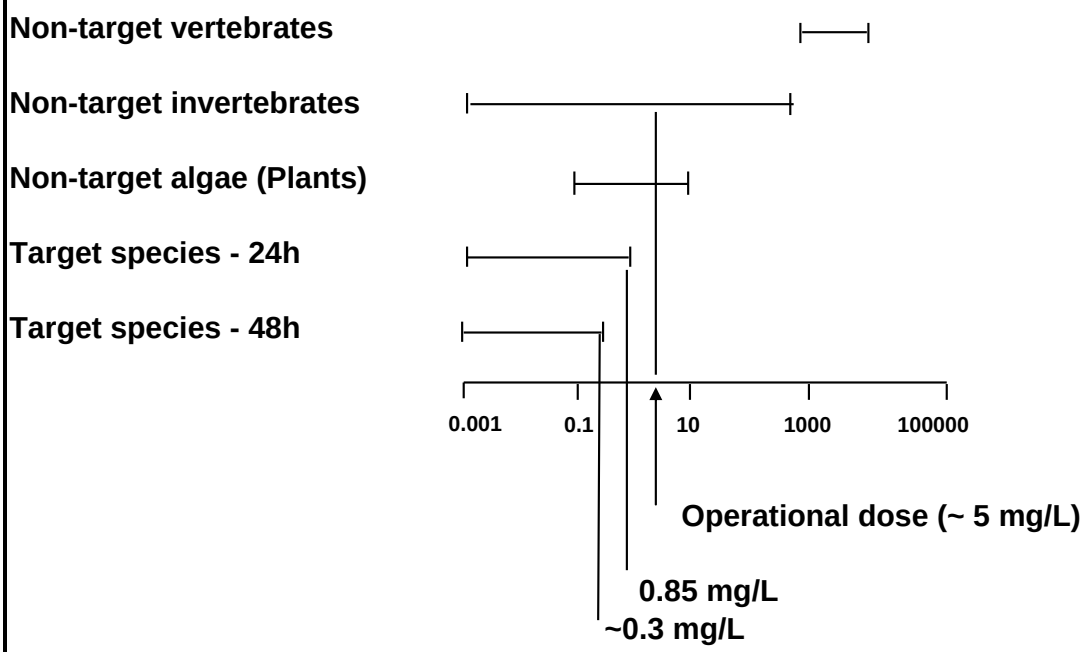

Table 4: AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
VERTEBRATES												
Acute non-target species												
Oncorhynchus mykiss (Steelhead trout)	24mm Fork length	24-h 100% Mortality	8.4-8.9	7.0-8.0	82-100% Sat.[2]	233		10,000	R U	S A	27	Teknar 1500 ITU/L
Salvelinus fontinalis (Brook trout)	0.76g Fry (Parr stage)	3-h LC_{50} 95% CI	6.8-7.5	7.0-9.0	90-95% Sat.[2]			5254 7881 X 10^3 ITU/L	F U	S A	12	Teknar
Salvelinus fontinalis (Brook trout)	18mm Fork length	48-h LC_{50}	8.4-8.9	7.0-8.0	82-100% Sat.[2]	233		2321 3,481,500 ITU/L	R U	S A	27	Teknar 1500 ITU/L
Salmo trutta (Brown trout)	22mm Fork length	48-h LC_{50}	8.4-8.9	7.0-8.0	82-100% Sat.[2]	233		1691 2,536,500 ITU/L	R U	S A	27	Teknar 1500 ITU/L
Salmo trutta (Brown trout)	15mm Fork length	48-h LC_{50}	8.4-8.9	7.0-8.0	82-100% Sat.[2]	233		1000 1.5 X10^6 ITU/L	R U	S A	27	Teknar 1500 ITU/L
Poecilia reticulata (Guppy)	2.5-3.0 cm Larvae	24-h LC_{50}						>1000 15 X 10^6 ITU/L	S U	S A	11	H-14
Fundulus heteroclitus (Mummichog)	mean length: 5.1 cn mean weight: 2.7 g	96-h LC_{50}	8	23.9	60-100% Sat.[2]			980 1176 x 10^3 ITU/L	R U	S A	22	Vectobac 1200 ITU/mg
Gambusia affinis (Mosquito fish)	7-14 days old	24-h		25				>1.0[1]	S U	S A	28	Bti liquid
VERTEBRATES												
Chronic non-target species												
Rana temporaria (Frog)	Tadpoles	Reduced growth & metamorphosis 43 Days						10[1] 15 X 10^4 ITU/L	S U	S C	14	H-14

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			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Gambusia affinis (Mosquito fish)	field populations at different Life stages	Increased populations						6kg/ha [1]			40	Vecto Bac G 200
INVERTEBRATES **Acute non-target species**												
Daphnia magna (Water Flea)	< 24-h old	24h-LC_{50}		25				626.6	S U	S A	28	Bti granule
Daphnia pulex (Water Flea)	< 24-h old	48h-LC_{50}		25				0.34	S U	S A	28	Bti granule
Daphnia pulex (Water Flea)	< 24-h old	48h-LC_{50}		25				0.0039	S U	S A	28	Bti liquid
Procambarus clarkii (Crayfish)	Immature 25-40 mm long	96h-LC_{50}					100	103.24 123888 ITU/mg	S U	S A	9	Bactimos FC 1200 ITU/mg
Chironomus tentans (Midge)	Larvae	24h-LC_{50}						46.6 9320 ITU/L	-	-	23	VectoBac G 200 ITU/mg
Synclita obliteralis (Moth Larvae)	Larvae	53.30% Mortality						0.02 300 ITU/L	S U	S A	6	H-14
Chironomus salinarius (Midge)	Late 3rd and early 4th instar larvae	48h-LC_{50}						4.46 15,610 ITU/L	S U	S A	20	Bactimos WP 3500 AAIU/mg
Chironomus salinarius (Midge)	Late 3rd and early 4th instar larvae	48h-LC_{50}						5.4 10,800 ITU/L	S U	S A	20	VectoBac WP 2000 AAIU/mg
Chironomus salinarius (Midge)	Late 3rd and early 4th instar larvae	48h-LC_{50}						14.63 21,945 ITU/L	S U	S A	20	Teknar FC 1500 AAIU/mg
Chironomus tepperi (Midge)	6-7 day-old 4th instar Larvae	48h-LC_{50}		25				0.245 735 ITU/L	S U	S A	34	VectoBac WDG 3000 ITU/mg

Table 4: **AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS**

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Chironomus tepperi (Midge)	10-14 day-old 4th instar Larvae	48h-LC_{50}		25				0.63 1890 ITU/L	S U	S A	34	VectoBac WDG 3000 ITU/mg
Chironomus thummi (Midge)	4th instar Larvae	24h-LC_{50}		20				0.77 6930 ITU/L	S U	S A	13	Bactimos PP 9000 ITU/mg
Chironomus thummi (Midge)	4th instar Larvae	48h-LC_{50}		20				0.33 2970 ITU/L	S U	S A	13	Bactimos PP 9000 ITU/mg
Chironomus riparius (Midge)	3rd & 4th instar Larvae	48h-EC_{50}		20		280	255	0.2	S U	S A	26	VectoBac G 200 ITU/mg
Psectrocladius psilopterus (Midge)	4th instar Larvae	24h-LC_{50}		20				1.17 12,285 ITU/L	S U	S A	13	Bactimos PP 10,500 ITU/mg
Psectrocladius psilopterus (Midge)	4th instar Larvae	48h-LC_{50}		20				0.57 5985 ITU/L	S U	S A	13	Bactimos PP 10,500 ITU/mg
Telmatoscopus albipunctatus (Sewage Moth fly)	4 day old Larvae	24h-LC_{50}		25				5.5	S U	S A	39	IPS-82 15000 ITU/mg
Tabanus triceps (Horsefiy) Instars	1st instar Larvae	24h-LC_{50}		28				0.308 4622 ITU/L	S U	S A	21	IPS-82-H14 15000 ITU/mg
Tabanus triceps (Horsefiy)	2nd instar Larvae	24h-LC_{50}		28				0.369 5533 ITU/L	S U	S A	21	IPS-82-H14 15000 ITU/mg
Tabanus triceps (Horsefiy)	3rd instar Larvae	24h-LC_{50}		28				0.413 6200 ITU/L	S U	S A	21	IPS-82-H14 15000 ITU/mg
Tabanus triceps (Horsefiy)	4th instar Larvae	24h-LC_{50}		28				0.471 7068 ITU/L	S U	S A	21	IPS-82-H14 15000 ITU/mg
Tabanus triceps (Horsefiy)	5th instar Larvae	24h-LC_{50}		28				0.528 7886 ITU/L	S U	S A	21	IPS-82-H14 15000 ITU/mg
Simulium tuberosum	Larvae	5h-LC_{50}						0.095	S U	S A	1	Vectobac, WP

Table 4: AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
(Blackfly)								189 ITU/L				2,000 ITU/mg
Phlebotomus papatasi (Sandfly)	Larvae	48h-LC_{50}						0.0026 39 ITU/L	S U	S A	32	IPS 82 15000 ITU/mg
MOSQUITOES												
Acute target species												
Culex pipiens molestus (Mosquito)	4 day old Larvae	24h-LC_{50}		25				0.33	S U	S A	39	IPS-82 15000 ITU/mg
Anopheles quadrimaculatus (Mosquito)	2nd & 3rd instar	48h-LC_{50}		25-27				0.0076	S U	S A	28	Bti liquid
Aedes albopictus **C** [4] Tigar Mosquito	Early 4th instar Larvae	24h-LC_{50}		26				0.181 905 ITU/L	S U	S A	7	Vectobac, TP 5,000 ITU/mg
Aedes albopictus **C** [4] Tigar Mosquito	Early 4th instar Larvae	24h-LC_{50}		26				0.849 1018.8 ITU/L	S U	S A	7	Bactimos, FC 1,200 ITU/mg
Aedes atropalpus **C** [4] (Mosquito)	4th instar Larvae	48h-LC_{50}	6.8-7.5	7.0-9.0	90-95% Sat.[2]			0.3075 461.2 ITU/L	S U	S A	12	Teknar HP-D 1500 AAU/mg
Aedes atropalpus **C** [4] (Mosquito)	Neonate Larvae	24h-LC_{50}		20-22				0.078 117 ITU/L	S U	S A	15	Teknar HP-D 1500 AAU/mg
Aedes aegypti (Mosquito)	4th instar Larvae	24h-LC_{50}		24			T A [3] 0	0.045	S U	S A	41	HD-968-S-1983
Aedes aegypti (Mosquito)	4th instar Larvae	24h-LC_{50}		24			T A [3] 1275	0.233	S U	S A	41	HD-968-S-1983
Anopheles arabiensis Malaria Vector Mosquito Instars	2nd instar Larvae	48h-LC_{50}						0.001 150 ITU/L	S U	S A	2	IPS-82 15000 ITU/mg
Anopheles arabiensis	3rd instar	48h-LC_{50}						0.0018	S U	S A	2	IPS-82

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SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
	Larvae							270 ITU/L				15000 ITU/mg
Aedes aegypti (Mosquito) Instars	2nd instar Larvae	24h-LC_{50}		28				0.06 36 ITU/L	S U	S A	19	600 ITU/mg
Aedes aegypti (Mosquito)	3rd instar Larvae	24h-LC_{50}		28				0.1 60 ITU/L	S U	S A	19	600 ITU/mg
Aedes aegypti (Mosquito)	4th instar Larvae	24h-LC_{50}		28				0.14 84 ITU/L	S U	S A	19	600 ITU/mg
Aedes aegypti (Mosquito)	4th instar Larvae	48h-LC_{50}		27				0.019 285 ITU/L	S U	S A	3	IPS-82 15000 ITU/mg
Aedes stimulans (Mosquito) Temperature	3rd instar Larvae	48h-LC_{50}		22				0.1 150 ITU/L	S U	S A	4	Teknar HP-D 1500 AAU/mg
Aedes stimulans (Mosquito)	3rd instar Larvae	48h-LC_{50}		4				0.2 300 ITU/L	S U	S A	4	Teknar HP-D 1500 AAU/mg
Aedes stimulans (Mosquito)	3rd instar Larvae	48h-LC_{50}		0				0.9 1350 ITU/L	S U	S A	4	Teknar HP-D 1500 AAU/mg
Aedes aegypti (Mosquito)	4th instar Larvae	24h-LC_{50}		25				0.8 160 ITU/L	S U	S A	5	Vectobac-G 200 ITU/mg
Anopheles quadrimaculatus (Mosquito)	4th instar Larvae	24h-LC_{50}					100	0.014 16.8 ITU/L	S U	S A	9	Bactimos FC 1200 ITU/mg
Culex salinarius (Mosquito)	4th instar Larvae	24h-LC_{50}					100	0.0062 7.44 ITU/L	S U	S A	9	Bactimos FC 1200 ITU/mg
Psorophora columbiae (Mosquito)	4th instar Larvae	24h-LC_{50}					100	0.0063 7.56 ITU/L	S U	S A	9	Bactimos FC 1200 ITU/mg

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SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Aedes aegypti (Mosquito)	4th instar Larvae	24h-LC_{50}		28-32				0.0027 3.24 ITU/L	S U	S A	10	BMP 144 2X 1200 ITU/mg
Anopheles stephensi (Mosquito)	Larvae	24h-LC_{50}						0.16 2400 ITU/L	S U	S A	11	H-14
Culex quinquefasciatus Southern House Mosquito	Larvae	24-LC_{50}						0.062 930 ITU/L	S U	S A	11	H-14
Aedes aegypti (Mosquito)	2nd instar Larvae	48h-LC_{50}		30				0.0065 96 ITU/L	S U	S A	25	IPS82 (H14) 15000 ITU/mg
Culex quinquefasciatus Southern House Mosquito	2nd instar Larvae	48h-LC_{50}		30				0.0038 57 ITU/L	S U	S A	25	IPS82 (H14) 15000 ITU/mg
Culex sitiens (Saltmarsh Mosquito)	3rd instar Larvae	48h-LC_{50}						0.019 57 ITU/L	S U	S A	29	VectoBac WG 3000 ITU/mg
Culex annulirostris (Freshwater Mosquito)	3rd instar Larvae	48h-LC_{50}						0.004 12 ITU/L	S U	S A	29	VectoBac WG 3000 ITU/mg
Culex quinquefasciatus Freshwater Mosquito	3rd instar Larvae	48h-LC_{50}						0.005 15 ITU/L	S U	S A	29	VectoBac WG 3000 ITU/mg
Culex tarsalis (Mosquito) Instars	2nd instar Larvae	24h-LC_{50}		28				0.15 90 ITU/L	S U	S A	19	Biopestide P 600 ITU/mg
Culex tarsalis	3rd instar Larvae	24h-LC_{50}		28				0.17 102 ITU/L	S U	S A	19	Biopestide P 600 ITU/mg
Culex tarsalis	4th instar Larvae	24h-LC_{50}		28				0.2 120 ITU/L	S U	S A	19	Biopestide P 600 ITU/mg
Aedes aegypti (Container-breeding Mosquito)	3rd instar Larvae	48h-LC_{50}						0.018 54 ITU/L	S U	S A	29	VectoBac WG 3000 ITU/mg

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			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Ochlerotatus vigilax (Saltmarsh Mosquito)	3rd instar Larvae	48h-LC_{50}						0.013 39 ITU/L	S U	S A	29	VectoBac WG 3000 ITU/mg
Ochlerotatus notoscriptus (Container-breeding Mosquito)	3rd instar Larvae	48h-LC_{50}						0.015 45 ITU/L	S U	S A	29	VectoBac WG 3000 ITU/mg
Anopholes gambiae (Malaria Mosquito)	3rd instar Larvae	24h-LC_{50}		23-30				0.021 57 ITU/L	S U	S A	30	VectoBac WDG 2700 ITU/mg
Anopholes gambiae (Malaria Mosquito)	3rd instar Larvae	24h-LC_{50}		23-30				0.006 60 ITU/L	S U	S A	30	Bactomos PP 10000 ITU/mg
Culex quinequefasciatus (Southern House Mosquito)	4th instar Larvae	24h-LC_{50}		27-28				0.009	S U	S A	31	Bti VS063-1 TP
Aedes aegypti (Mosquito)	4th instar Larvae	24h-LC_{50}		27-28				0.014	S U	S A	31	Bti VS063-1 TP
Aedes aegypti (Mosquito)	3rd instar Larvae	24h-LC_{50}						0.00174 2.09 ITU/L	S U	S A	33	Teknar HP-D 1200 ITU/mg
Anopheles stephensi (Mosquito)	3rd instar Larvae	24h-LC_{50}						0.00213 2.56 ITU/L	S U	S A	33	Teknar HP-D 1200 ITU/mg
Culex quinquefasciatus (Southern House Mosquito)	3rd instar Larvae	24h-LC_{50}						0.00277 3.32 ITU/L	S U	S A	33	Teknar HP-D 1200 ITU/mg
Aedes aegypti (Dengue Fever Mosquito)	Late 3rd or early 4th instar larvae	24h-LC_{50}		27				0.0638 76.56 ITU/L	S U	S A	35	Vectbac 12AS 1200 ITU/mg
Aedes aegypti (Dengue Fever Mosquito)	Late 3rd or early 4th instar larvae	24h-LC_{50}		27				0.145 174 ITU/L	S U	S A	35	BMP 144-2x 1200 ITU/mg
Anopheles stephensi (Malaria Mosquito)	Late 3rd or early 4th instar larvae	24h-LC_{50}		27				0.14 168 ITU/L	S U	S A	35	Vectbac 12AS 1200 ITU/mg

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SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Anopheles stephensi (Malaria Mosquito)	Late 3rd or early 4th instar larvae	24h-LC_{50}		27				0.81 972 ITU/L	S U	S A	35	BMP 144-2x 1200 AA ITU/mg
Aedes aegypti (Mosquito)	3rd instar larvae	LT_{50} (min)		17.8				261.2 2,500 ITU/L	S U	S A	42	Vectbac 12AS 1200 AA ITU/mg
Aedes aegypti	3rd instar larvae	LT_{50} (min)		17.8				235 5000 ITU/L	S U	S A	42	Vectbac 12AS 1200 AA ITU/mg
Aedes aegypti	Early 4th instar larvae	LT_{50} (min)		17.8				391.4 2,500 ITU/L	S U	S A	42	Vectbac 12AS 1200 AA ITU/mg
Aedes aegypti	Early 4th instar larvae	LT_{50} (min)		17.8				298 5000 ITU/L	S U	S A	42	Vectbac 12AS 1200 AA ITU/mg
Aedes aegypti	Late 4th instar larvae	LT_{50} (min)		17.8				362.1 5000 ITU/L	S U	S A	42	Vectbac 12AS 1200 AA ITU/mg
Aedes aegypti (Mosquito) Product Potency	3rd instar Larvae	24h-LC_{50}		25				0.42 504 ITU/L	S U	S A	37	Cybate 1200 ITU/mg
Aedes aegypti	3rd instar Larvae	24h-LC_{50}		25				0.14 168 ITU/L	S U	S A	37	Teknar 1200 ITU/mg
Aedes aegypti	3rd instar Larvae	24h-LC_{50}		25				0.2 240 ITU/L	S U	S A	37	VectoBac 12AS 1200 ITU/mg
Aedes notoscriptus (Mosquito) Product Potency	3rd instar Larvae	24h-LC_{50}		25				0.25 300 ITU/L	S U	S A	37	Cybate 1200 ITU/mg
Aedes notoscriptus	3rd instar Larvae	24h-LC_{50}		25				0.11 132 ITU/L	S U	S A	37	Teknar 1200 ITU/mg
Aedes notoscriptus	3rd instar Larvae	24h-LC_{50}		25				0.11 132 ITU/L	S U	S A	37	VectoBac 12AS 1200 ITU/mg

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SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Aedes vigilax (Mosquito) Product Potency	3rd instar Larvae	24h-LC_{50}		25				0.04 48 ITU/L	S U	S A	37	Cybate 1200 ITU/mg
Aedes vigilax	3rd instar Larvae	24h-LC_{50}		25				0.02 24 ITU/L	S U	S A	37	Teknar 1200 ITU/mg
Aedes vigilax	3rd instar Larvae	24h-LC_{50}		25				0.03 36 ITU/L	S U	S A	37	VectoBac 12AS 1200 ITU/mg
Aedes camptorhynchus (Mosquito) Product Potency	3rd instar Larvae	24h-LC_{50}		25				0.07 84 ITU/L	S U	S A	37	Cybate 1200 ITU/mg
Aedes camptorhynchus	3rd instar Larvae	24h-LC_{50}		25				0.05 60 ITU/L	S U	S A	37	Teknar 1200 ITU/mg
Aedes camptorhynchus	3rd instar Larvae	24h-LC_{50}		25				0.04 48 ITU/L	S U	S A	37	VectoBac 12AS 1200 ITU/mg
Mosquito Instars - VectoBac 12AS												
Aedes taeniorhynchus (Saltwater Mosquito)	2nd instar Larvae	24h-LC_{50}		25				0.022 26.4 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Aedes taeniorhynchus	3rd instar Larvae	24h-LC_{50}		25				0.09 108 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Aedes taeniorhynchus	4th instar Larvae (N)	24h-LC_{50}		25				0.164 196.8 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Aedes taeniorhynchus	4th instar Larvae (M)	24h-LC_{50}		25				0.208 249.6 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Aedes taeniorhynchus	4th instar Larvae (L)	24h-LC_{50}		25				0.317 380.4 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Culex nigripalpus	2nd instar	24h-LC_{50}		25				0.016	S U	S A	16	VectoBac 12AS

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SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
(Freshwater Mosquito)	Larvae							19.2 ITU/L				1200 ITU/mg
Culex nigripalpus	3rd instar Larvae	24h-LC_{50}		25				0.053 63.6 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Culex nigripalpus	4th instar Larvae (N)	24h-LC_{50}		25				0.088 105.6 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Culex nigripalpus	4th instar Larvae (M)	24h-LC_{50}		25				0.131 157.2 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Culex nigripalpus	4th instar Larvae (L)	24h-LC_{50}		25				0.165 198 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Mosquito Instars - VectoBac TP												
Aedes taeniorhynchus (Saltwater Mosquito)	2nd instar Larvae	24h-LC_{50}		25				0.005 25 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Aedes taeniorhynchus	3rd instar Larvae	24h-LC_{50}		25				0.012 60 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Aedes taeniorhynchus	4th instar Larvae (N)	24h-LC_{50}		25				0.026 130 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Aedes taeniorhynchus	4th instar Larvae (M)	24h-LC_{50}		25				0.036 180 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Aedes taeniorhynchus	4th instar Larvae (L)	24h-LC_{50}		25				0.032 160 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Culex nigripalpus (Freshwater Mosquito)	2nd instar Larvae	24h-LC_{50}		25				0.002 10 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Culex nigripalpus	3rd instar	24h-LC_{50}		25				0.005	S U	S A	16	VectoBac TP

Table 4: **AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS**

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
	Larvae							25 ITU/L				5000 ITU/mg
Culex nigripalpus	4th instar Larvae (N)	24h-LC_{50}		25				0.009 45 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Culex nigripalpus	4th instar Larvae (M)	24h-LC_{50}		25				0.01 50 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Culex nigripalpus	4th instar Larvae (L)	24h-LC_{50}		25				0.017 85 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Temperature and multible Instars - VectoBac 12AS												
Culex nigripalpus (Freshwater Mosquito)	3rd instar Larvae	24h-LC_{50}		15				0.094 112.8 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Culex nigripalpus	3rd instar Larvae	24h-LC_{50}		25				0.074 88.8 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Culex nigripalpus	3rd instar Larvae	24h-LC_{50}		35				0.066 79.2 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Culex nigripalpus	4th instar Larvae	24h-LC_{50}		15				0.152 182.4 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Culex nigripalpus	4th instar Larvae	24h-LC_{50}		25				0.139 166.8 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Culex nigripalpus	4th instar Larvae	24h-LC_{50}		35				0.14 168 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Aedes taeniorhynchus (Saltwater Mosquito)	3rd instar Larvae	24h-LC_{50}		15				0.072 86.4 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Aedes taeniorhynchus	3rd instar	24h-LC_{50}		25				0.056	S U	S A	16	VectoBac 12AS

Table 4: AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
	Larvae							67.2 ITU/L				1200 ITU/mg
Aedes taeniorhynchus	3rd instar Larvae	24h-LC_{50}		35				0.052 62.4 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Aedes taeniorhynchus	4th instar Larvae	24h-LC_{50}		15				0.292 350.4 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Aedes taeniorhynchus	4th instar Larvae	24h-LC_{50}		25				0.202 242.4 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Aedes taeniorhynchus	4th instar Larvae	24h-LC_{50}		35				0.224 268.8 ITU/L	S U	S A	16	VectoBac 12AS 1200 ITU/mg
Temperature and multible Instars - VectoBac TP												
Culex nigripalpus (Freshwater Mosquito)	3rd instar Larvae	24h-LC_{50}		15				0.018 90 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Culex nigripalpus	3rd instar Larvae	24h-LC_{50}		25				0.011 55 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Culex nigripalpus	3rd instar Larvae	24h-LC_{50}		35				0.006 30 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Culex nigripalpus	4th instar Larvae	24h-LC_{50}		15				0.017 85 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Culex nigripalpus	4th instar Larvae	24h-LC_{50}		25				0.013 65 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Culex nigripalpus	4th instar Larvae	24h-LC_{50}		35				0.016 80 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg

Table 4: AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Aedes taeniorhynchus (Saltwater Mosquito)	3rd instar Larvae	24h-LC_{50}		15				0.009 45 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Aedes taeniorhynchus	3rd instar Larvae	24h-LC_{50}		25				0.01 50 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Aedes taeniorhynchus	3rd instar Larvae	24h-LC_{50}		35				0.005 25 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Aedes taeniorhynchus	4th instar Larvae	24h-LC_{50}		15				0.049 245 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Aedes taeniorhynchus	4th instar Larvae	24h-LC_{50}		25				0.028 140 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Aedes taeniorhynchus	4th instar Larvae	24h-LC_{50}		35				0.036 180 ITU/L	S U	S A	16	VectoBac TP 5000 ITU/mg
Product IPS-78 *Aedes cantans* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.203	S U	S A	18	15000 ITU/mg
Product IPS-78 *Aedes cantans* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.157	S U	S A	18	
Product APG-6108 *Aedes cantans* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.32	S U	S A	18	
Product APG-6108 *Aedes cantans* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.22	S U	S A	18	
Product R-153-78 *Aedes cantans* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.073	S U	S A	18	

Table 4: **AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS**

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Product R-153-78 *Aedes cantans* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.064	S U	S A	18	
Product TEKNAR *Aedes cantans* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.347	S U	S A	18	
Product TEKNAR *Aedes cantans* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.229	S U	S A	18	
Product IPS-78 *Aedes communis* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.118	S U	S A	18	
Product IPS-78 *Aedes cantans* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.074	S U	S A	18	
Product APG-6108 *Aedes communis* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.164	S U	S A	18	
Product APG-6108 *Aedes communis* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.088	S U	S A	18	
Product R-153-78 *Aedes communis* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.038	S U	S A	18	
Product R-153-78 *Aedes communis* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.02	S U	S A	18	

Table 4: **AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS**

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Product TEKNAR *Aedes communis* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.144	S U	S A	18	
Product TEKNAR *Aedes communis* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.071	S U	S A	18	
Product IPS-78 *Aedes punctor* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.2	S U	S A	18	
Product IPS-78 *Aedes punctor* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.113	S U	S A	18	
Product APG-6108 *Aedes punctor* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.23	S U	S A	18	
Product APG-6108 *Aedes punctor* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.11	S U	S A	18	
Product R-153-78 *Aedes punctor* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.069	S U	S A	18	
Product R-153-78 *Aedes punctor* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.032	S U	S A	18	
Product TEKNAR *Aedes punctor* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.228	S U	S A	18	

Table 4: AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Product TEKNAR *Aedes punctor* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.138	S U	S A	18	
Product IPS-78 *Aedes vexans* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.106	S U	S A	18	
Product IPS-78 *Aedes vexans* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.088	S U	S A	18	
Product APG-6108 *Aedes vexans* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.082	S U	S A	18	
Product APG-6108 *Aedes vexans* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.054	S U	S A	18	
Product R-153-78 *Aedes vexans* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.051	S U	S A	18	
Product R-153-78 *Aedes vexans* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.03	S U	S A	18	
Product TEKNAR *Aedes vexans* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.242	S U	S A	18	
Product TEKNAR *Aedes vexans* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.136	S U	S A	18	

Table 4: **AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS**

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Product IPS-78 *Aedes cinereus* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.088	S U	S A	18	
Product IPS-78 *Aedes cinereus* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.07	S U	S A	18	
Product APG-6108 *Aedes cinereus* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.092	S U	S A	18	
Product APG-6108 *Aedes cinereus* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.053	S U	S A	18	
Product R-153-78 *Aedes cinereus* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.034	S U	S A	18	
Product R-153-78 *Aedes cinereus* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.018	S U	S A	18	
Product TEKNAR *Aedes cinereus* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.118	S U	S A	18	
Product TEKNAR *Aedes cinereus* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.068	S U	S A	18	
Product IPS-78 *Culex pipiens pipiens* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.155	S U	S A	18	

Table 4: **AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS**

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Product IPS-78 *Culex pipiens pipiens* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.129	S U	S A	18	
Product APG-6108 *Culex pipiens pipiens* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.123	S U	S A	18	
Product APG-6108 *Culex pipiens pipiens* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.105	S U	S A	18	
Product R-153-78 *Culex pipiens pipiens* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.08	S U	S A	18	
Product R-153-78 *Culex pipiens pipiens* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.038	S U	S A	18	
Product TEKNAR *Culex pipiens pipiens* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.09	S U	S A	18	
Product TEKNAR *Culex pipiens pipiens* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.072	S U	S A	18	
Product IPS-78 *Culex pipiens molestus* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.067	S U	S A	18	
Product IPS-78 *Culex pipiens molestus* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.051	S U	S A	18	

Table 4: **AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS**

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Product APG-6108 *Culex pipiens molestus* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.104	S U	S A	18	
Product APG-6108 *Culex pipiens molestus* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.08	S U	S A	18	
Product R-153-78 *Culex pipiens molestus* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.016	S U	S A	18	
Product R-153-78 *Culex pipiens molestus* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.015	S U	S A	18	
Product TEKNAR *Culex pipiens molestus* (Mosquito)	4th instar Larvae	24h-LC_{50}		22				0.16	S U	S A	18	
Product TEKNAR *Culex pipiens molestus* (Mosquito)	4th instar Larvae	48h-LC_{50}		22				0.105	S U	S A	18	
Product IPS - 78 *Culex pipiens* (Mosquito)	2nd instar Larvae	48h-LC_{50}		27				0.037	S U	S A	24	Vectobac WP 2000 ITU/mg
ABG-6108-II *Culex pipiens* (Mosquito)	4th instar Larvae	48h-LC_{50}		27				0.033	S U	S A	24	
ABG-6108-II *Culex restuans* (Mosquito)	4th instar Larvae	48h-LC_{50}		27				0.072	S U	S A	24	

Table 4: **AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS**

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
R-153-78 *Culex restuans* (Mosquito)	4th instar Larvae	48h-LC_{50}		27				0.098	S U	S A	24	1.5 x 10^{8}sp/mg RB Biochem Lab
R-153-78 *Aedes vexans* (Mosquito)	3rd instar Larvae	48h-LC_{50}		21				0.034	S U	S A	24	
IPS-78 *Aedes simulans* (Mosquito)	3rd instar Larvae	48h-LC_{50}		21				0.081	S U	S A	24	
IPS-78 *Aedes aegypti* (Mosquito)	4th instar Larvae	48h-LC_{50}		26				0.343	S U	S A	24	
Bactimos *Aedes triseriatus* (Mosquito)	3rd instar Larvae	48h-LC_{50}		23				0.333	S U	S A	24	3500 ITU/mg
MOSQUITOES **Chronic target species**												
Ochlerotatus squamiger (Mosquito)	4th instar larvae	72h-LD_{50}		10				0.1472 441.6 ITU/L	S U	S A	36	VectoBac WDG 3000 ITU/mg
Ochlerotatus squamiger (Mosquito)	4th instar larvae	72h-LD_{50}		6				0.226 1130 ITU/L	S U	S A	36	VectoBac TP 5000 ITU/mg
Ochlerotatus squamiger (Mosquito)	4th instar larvae	72h-LD_{50}		10				0.1224 612 ITU/L	S U	S A	36	VectoBac TP 5000 ITU/mg

Table 4: **AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS**

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					
Ochlerotatus squamiger (Mosquito)	4th instar larvae	72h-LD_{50}		14				0.0903 451.5 ITU/L	S U	S A	36	VectoBac TP 5000 ITU/mg
INVERTEBRATES												
Chronic non-target species												
OTHER ORGANISMS												
Closterium sp. (Green algae)	Cells per mL	7 Day-48% Reduced Den.,Growth & Prod.	7.5-8.5	20-35	14-18			12 2404 ITU/L	S, U, MI	S, C	38	VectoBac G 200 ITU/mg
Chlorella sp. (Green algae)	Cells per mL	2 Day-47% Reduced Den.,Growth & Prod.	7.5-8.5	20-35	14-18			12 2404 ITU/L	S, U, MI	S, C	38	VectoBac G 200 ITU/mg
Closterium sp. (Green algae)	Cells per mL	3 Day-99% Reduced Den.,Growth & Prod.	7.5-9.0	13-26	14-23			0.15 600 ITU/L	S, U, MI	S, C	38	VectoBac WDG 4000 ITU/mg
Chlorella sp. (Green algae)	Cells per mL	7 Day-98% Reduced Den.,Growth & Prod.	7.5-9.0	13-25	14-23			0.15 600 ITU/L	S, U, MI	S, C	38	VectoBac WDG 4000 ITU/mg

Table 4: **AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS**

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					

KEY (1):

F = flowthrough
R = renewed static
S = static
M = measured toxicant concentration
U = unmeasured toxicant concentration
NP = not present
MI = microcosms

KEY (2):

P = primary
S = secondary
A = acute toxicity
C = chronic toxicity

KEY (3):

1. Barton *et al.* 1991
2. Seyoum and Abate 1997
3. Misch *et al.* 1992
4. Wa ker 1995
5. Chui *et al* . 1995
6. Haag and Buckingham 1991
7. Ali *et al.* 1995
8. Ross *et al* . 1994
9. Holck and Meek 1987
10. Seleena *et al.* 1996
11. Mittal *et al.* 1994
12. Fortin *et al* . 1986
13. Yiallouros *et al.* 1999
14. Paulov 1987
15. Tousignant *et al.* 1992
16. Nayar *et al.* 1999
17. Arshad *et al.* 1995
18. Rettich 1983
19. Qadri *et al* . 1990
20. Arshad *et al* . 1985
21. Saraswathi and Ranganathan 1996
22. Lee and Scott 1989
23. Liber *et al.* (1998)
24. Wraight *et al.* 1987
25. Sun *et al.* 1996
26. Charbonneau *et al.* 1994
27. Wipfli *et al.* 1994
28. Milam et al. 2000
29. Russell et al. 2003
30. Fillinger et al. 2003
31. Zahiri *et al.* 2004

Footnotes:
[1] - No mortality at this concentration
[2] - Saturation
[3] - T. A. = tannic acid
[4] - C- Species found in Canada

Table 4: AQUATIC TOXICITY DATA TABLE FOR BACILLUS THURINGIENSIS VAR. ISRAELENSIS

SPECIES	LIFE STAGE	RESPONSE	TEST CONDITIONS					EFFECT CONC (mg/L)	DATA CODES KEY(1)	DATA QUALITY KEY(2)	DATA REF KEY(3)	Bti PROD POTENCY
			pH	TEMP (°C)	DO (mg/L)	ALK. (mg/L	HARD (mg/L)					

32. Wahba *et al.* 1999
33. Gunasekaran et al. 2004
34. Stevens et al. 2004
35. Mittal *et al* . 2001
36. Christiansen *et al* . 2004
37. Brown *et al.* 2001
38. Su and Mulla 1999
39. Saitoh *et al.* 1996
40. Kramer *et al.* 1988
41. Lord and Undeen 1990
42. de Andrade and Modolo 1999